Correlation of synovial fluid cytokine levels with histological and clinical parameters of primary and revision total hip and total knee replacements

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ABSTRACT – We retrieved synovial tissue and fluid samples from patients undergoing primary total hip replacement (THR) (n 15), revision of aseptically loose THR (n 12), primary total knee replacement (TKR) (n 13) and revision of aseptically loose TKR (n 6). Several histological parameters were assessed on a relative scale of 1–4. Primary TJRs were clinically evaluated for degree of osteoarthrosis. Revision TJRs were assessed for migration of the implant, gross loosening and the degree of radiolucency. Cytokine levels in synovial fluid were determined with ELISA.

All cytokines were significantly higher in revision TJRs than in primary replacements, as were the degree of macrophage and giant cell infiltration. We found no relationship between any clinical variable and the levels of any cytokine, but migration of the implant was related to the presence of PE debris. A significant correlation was seen between the presence of macrophages and the levels of IL-1β, IL-8 and IL-10, but not IL-6. No differences were noted between hips and knees for any of the variables, except in the levels of IL-6, where higher levels were found in THRs. These results suggest a unique role for IL-6 that requires further investigation.

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Aseptic loosening of total joint replacement is often accompanied by focal osteolysis and is characterized by the presence of a fibrous membrane between the implant and bone (interface) (Jasty et al. 1984). Inflammatory cells in this interface secrete proinflammatory mediators into the surrounding tissue and joint fluid. Several of these mediators are cytokines, such as interleukin-1β (IL-1β), IL-6, IL-8 and tumour necrosis factor-α (TNF-α) that have the capacity to stimulate bone resorption. The presence of all these cytokines in interface tissue has been confirmed by mRNA or immunohistochemical analysis (Al Saffar et al. 1995, Ishiguro et al. 1997) and higher levels of TNF-α (Xu et al. 1996), IL-8 (Lassus et al. 2000) and macrophage-colony stimulating factor (M-CSF) (Xu et al. 1997) were found in interface membranes around loose prostheses than in stable implants or in interface tissue than in control synovium. Furthermore, the presence of osteolysis around cemented and uncemented implants has been positively correlated with expression of some of these cytokines (Murray and Rushton 1990, Shanbhag et al. 1994). The anti-inflammatory cytokine IL-10 has also been found in periprosthetic tissue (Hsu et al. 1997).

Periprosthetic wear debris has been shown to stimulate the release of cytokines by macrophages in vitro and some authors have reported variations in the responses stimulated by different particulate materials (Rae 1975, Haynes et al. 1993, Shanbhag et al. 1995). The nature and amount of wear debris may therefore influence the histological features and aggressiveness of the inflammatory response found at aseptically loose implants.

We studied the relationship between histological, clinical and radiographical features of total joint arthroplasties (hips and knees) revised due to aseptic loosening and levels of IL-1β, IL-6, IL-8 and IL-10 in synovial fluid from these patients.

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Table 1. Details of the patients in each group and of the revised prostheses

	Hip primary	Knee primary	Hip revision	Knee revision
Total samples	15	13	12	6
Age range	26–82	62–84	46–86	48–79
Male	5	5	5	4
Female	10	8	7	2
OA	15	13	12	6
Mean implant duration, months (range)			121 (18–240)	42 (17–84)
Cemented			7	4
Uncemented			1	0
Metal/PE			8	4
Unknown			4	2

Synovial fluid and membranes from patients undergoing primary total joint replacement were used for comparison.

Patients and methods

Specimen retrieval

We retrieved tissue and synovial fluid samples from 4 groups of patients undergoing surgery for primary total hip replacements (n 15), revision of aseptically loose total hip replacements (n 12), primary total knee replacements (n 13) and revision of aseptically loose total knee replacements (n 6) (Table 1). No evidence of infection was observed during implant retrieval. The indication for primary TJR in all patients was OA.

Clinical assessment

Patients undergoing primary hip replacements were graded for degree of OA on a scale of 1–10 by assessing the following parameters: narrowing of the superior and minimum joint spaces, osteophyte formation, presence of cysts and bone loss. Similarly, those undergoing primary knee replacements were graded for degree of OA on the basis of narrowing of the minimum joint space, osteophyte formation, bone loss, sclerosis and alignment of the tibia using degree of valgus.

Migration of, or a grossly loose implant, if present, were recorded for those undergoing revision surgery. Radiolucency around the implant was graded on a scale of 0–3, based on the method of Harris et al. (1982), where 0 equals no radiolucent areas, 1 represents 1–50% of the implant surrounded by a radiolucent line, 2 equals 51–99% and 3 is an implant completely surrounded by a radiolucent area.

Histological assessment

Formalin-fixed, paraffin-embedded samples of synovial membrane from primary TJRs and periprosthetic tissue from revision of TJRs were sectioned (6 μm) and stained with hematoxylin and eosin using routine procedures.

A blinded observer graded a section of each sample for 10 features on a scale of 1–4, where 1 is absent and 4 is obvious, based on previously described methods (Hutton et al. 1987, Rooney et al. 1988, Kahle et al. 1992). These features were hyperplasia and hypertrophy of the synovial lining or synovial-like lining, amount of macrophages, T lymphocytes, foreign body giant cells (FBGC), blood vessels, polyethylene (PE) debris, metal debris, other wear debris (including polymethylmethacrylate (PMMA) particles) and fibrous tissue. Hyperplasia and hypertrophy of the lining were evaluated in three high-power fields (HPF × 400) per section and all other features were assessed in 17 HPF per section, as recommended by Bresnihan et al. (1998). The mean score of the HPFs for each of the 10 features was calculated for use in further analyses.

Cytokine assays

Synovial fluid samples were centrifuged at 2000 g for 10 min following collection, to remove cells and other debris. They were treated with 3000 IU/mL hyaluronidase (Sigma Aldrich, U.K.) for 60 min at room temperature and stored at –70 °C.

Prior to use, all samples were allowed to reach room temperature.

Levels of the cytokines in synovial fluid were measured using quantitative, non-competitive, sandwich ELISAs (Endogen, Ma, USA). The range of detectable IL-1β and IL-6 was 10.24–400 pg/mL with an assay sensitivity of < 1 pg/mL. The measurable ranges of IL-8 and IL-10 were 25.6–1000 pg/mL and 15.36–600 pg/mL, respectively, with sensitivities of < 2 pg/mL and < 3 pg/mL.

Statistics

The presence of an interaction between hip and knee groups and primary and revision groups was investigated within a multiple regression model. It was found that one grouping variable did not affect the other, therefore analysis was performed using tests for independent samples and no correction for the other grouping variable was necessary.

Investigations of differences in the histological variables between hip and knee groups and between primary and revision groups were performed using the Kruskal-Wallis test for independent samples. When considering differences in the cytokine levels between these groups, the data was logarithmically transformed to achieve normality and then tested using linear regression analysis.

A Pearson correlation test was used to investigate the relationship between cytokines as the data was normally distributed following logarithmic transformation. Hypotheses of relationships between the other clinical and histological variables were formed and then tested using a nonparametric Spearman's rho correlation test.

Kruskal-Wallis analysis was also used to investigate relationships between wear debris and cytokine levels with the presence of gross lossening or migration of the implant.

Results

Histology

Few samples had hyperplasia or hypertrophy of the synovial (primary cases; 6 and 3, respectively) or synovial-like lining cells (revision cases; 1 case of each). Furthermore these changes were distinct in sections from only 2 primary TJRs.

The degree of cellular infiltrate was much greater in periprosthetic tissue than in synovial tissue where there were large areas containing only fibrous connective tissue. Macrophages were the main cell type in all cases. Although 69% of all sections contained some lymphocytes, only 3 of these (2 primary, 1 revision) had levels greater than 2 on the relative scale. Lymphocytes were mainly found in focal aggregates that were often perivascular. Indeed, T-cell infiltrate correlated positively with the amount of blood vessels present in the tissue ($r_s = 0.35$, $p = 0.04$).

The presence of FBGC in the sections was dramatically increased in interface tissue. Although only 18% of synovial cases contained any FBGC, this figure increased to 86% in periprosthetic tissue.

All samples of interface from revisions contained some PE debris, but metal particles were found in just 4 cases. Only one of these contained heavy metal contamination and these particles were small and round in appearance. By contrast, large, shard-like particles of PE were often seen and were associated with FBGC. Other types of particles found in the sections were of PMMA, which is seen as empty spaces following processing, and zirconium.

Hip/knee

Of all the variables measured, whether histological, clinical or cytokine levels, only the synovial fluid levels of IL-6 were significantly different between the hip and knee. Higher levels were found in the fluid from hip joints than from knees (Table 2).

Primary/revision

The degree of macrophage and FBGC infiltration was significantly greater in the interface membrane from loose implants than in synovial tissue. We found no difference in the levels of hyperplasia or hypertrophy of the synovial or synovial-like lining cells, or in the degree of lymphocyte infiltration and neovascularization. Significantly greater levels of fibrosis were present in synovium than in periprosthetic tissue. The levels of all four cytokines tested were significantly higher in synovial fluid from revision operations than in synovial fluid from primary operations (Table 2).

Table 2. Median grade of several histological and clinical features and the mean (following logarithmic transformation) levels of cytokines found in synovial fluid from patients undergoing primary or revision total joint replacements

	Hip	Knee	P-value [a]	Primary	Revision	P-value [b]
Histological [c]						
Hyperplasia	1.00	1.00	0.5	1.00	1.00	0.6
Hypertrophy	1.00	1.00	0.3	1.00	1.00	0.1
Macrophages	2.56	2.38	0.3	2.29	3.88	0.001
Lymphocytes	1.09	1.21	0.2	1.21	1.06	0.1
FBGC	1.06	1.00	0.2	1.00	1.41	<0.001
Blood vessels	2.42	2.55	0.7	2.45	2.41	0.6
Fibrosis	3.26	3.62	0.3	3.79	3.00	0.004
Cytokines [d]						
IL-1β (pg/mL)	1.13	0.60	0.06	0.60	1.39	0.004
IL-6 (pg/mL)	5.03	3.81	0.01	4.05	5.28	0.01
IL-8 (pg/mL)	6.06	5.27	0.1	4.82	6.97	<0.001
IL-10 (pg/mL)	3.43	2.96	0.09	2.81	3.86	<0.001
Clinical [c]						
OA score	6.00	6.42	0.6			
Lucency	3.00	2.00	0.3			

[a] P-value refers to significance of difference between hip and knee cases (n 27 and n 19, respectively).
[b] P-value refers to significance of difference between primary and revision cases (n 28 and n 18, respectively).
[c] Tests performed using Kruskal-Wallis analysis.
[d] Tests performed using linear regression analysis.

Table 3. Correlation between specific cellular infiltration in periprosthetic tissue and synovial fluid cytokine levels with the presence of wear debris in the tissue (n 46)

	PE debris		Metal debris		Other debris		Total wear	
	r_s	P-value	r_s	P-value	r_s	P-value	r_s	P-value
Macrophages	0.619	<0.001	0.336	0.05	0.345	0.04	0.666	<0.001
FBGC	0.804	<0.001	0.417	0.01	0.365	0.03	0.809	<0.001
Lymphocytes	–0.187	0.281	0.048	0.8	–0.380	0.02	–0.203	0.2
IL-1β	0.475	0.004		0.7	0.338	0.05	0.416	0.01
IL-6	0.400	0.017	0.07	0.7	0.266	0.1	0.379	0.03
IL-8	0.741	<0.001	0.404	0.02	0.433	0.01	0.726	<0.001
IL-10	0.620	<0.001	0.143	0.4	0.209	0.2	0.604	<0.001
Implant migration [a]	4.349	0.037	0.000	1.0	0.730	0.4	3.403	0.07

[a] Analysis involving implant migration involved a Kruskal-Wallis test, therefore Chi-square values are shown.

Presence of particulate material

We found a positive correlation between the presence of both macrophages and FBGC, and the total amount of wear debris and each type of particulate seen in periprosthetic tissue. This correlation, however, was stronger and more significant with PE than with metal or 'other' wear debris. The lymphocyte infiltrate did not correlate with the total amount of wear debris, PE or metal, but the presence of 'other' particulates seemed to reduce the number of lymphocytes (Table 3).

No relationship was detected between the degree of radiographic lucency, the presence of gross loosening or migration of the prosthesis and the total amount of particulate material (data not shown), but migration of the implant was associated with

Table 4. Correlation between several parameters and the cytokine levels in synovial fluid (n 46)

	IL-1β		IL-6		IL-8		IL-10	
	r_s/r	P-value	r_s/r	P-value	r_s/r	P-value	r_s/r	P-value
Macrophages	0.383	0.02	0.325	0.06	0.474	0.005	0.563	<0.001
FBGC	0.471	0.004	0.389	0.02	0.773	<0.001	0.626	<0.001
Lymphocytes	0.001	1.0	0.106	0.5	–0.245	0.2	0.096	0.6
IL-β			0.394	0.007	0.518	<0.001	0.354	0.02
IL-6	0.394	0.007			0.407	0.006	0.741	<0.001
IL-8	0.518	<0.001	0.407	0.006			0.564	<0.001
IL-10	0.354	0.02	0.741	<0.001	0.564	<0.001		

Analysis involving histological variables was performed using Spearman's correlation (r_s) and relationships between cytokines was investigated using a Pearson correlation (r)

the presence of PE debris (p = 0.04).

Cytokine levels

We found a positive correlation between the presence of macrophages and FBGC with the levels of IL-1β, IL-8 and IL-10 in synovial fluid. However, the correlation with IL-6 was weaker and did not reach significance in relation to the number of macrophages. No relationship was seen between the levels of any of the cytokines and lymphocyte infiltration or any of the clinical parameters (Table 4).

The total amount of wear present in periprosthetic tissue and the amount of PE debris were positively correlated to the levels of all 4 cytokines in synovial fluid, but IL-1β was also affected by metal debris and IL-8 by both metal and 'other' types of particulates (Table 3). The levels of all cytokines in synovial fluid were positively correlated with each other (Table 4).

Discussion

We found statistically significant increases in the levels of IL-1β, IL-6, IL-8 and IL-10 in synovial fluid from TJRs requiring revision compared to those from patients undergoing primary joint replacement for OA. Such increases in cytokine levels presumably reflect the greater degree of inflammatory reaction in aseptic loosening of prostheses as compared with OA and indeed, a significant increase in the macrophage and FBGC infiltrates and a significant decrease in fibrous tissue was seen in periprosthetic tissue in this study. The presence of 'other' types of wear debris in the tissue had a negative effect on the size of the lymphocyte population. This may indicate that zirconia or PMMA particulates inhibit lymphocyte recruitment, or perhaps that metal and PE debris are more immunogenic than these particles.

The number of macrophages and FBGC in periprosthetic and synovial tissue correlated positively with the synovial fluid levels of IL-1β, IL-8 and IL-10 suggesting that these cells are primarily responsible for the production of these cytokines. Only the presence of FBGCs, however, correlated with the synovial fluid levels of IL-6. This could indicate that other cell types are secreting IL-6, for example, endothelial cells, fibroblasts or T-lymphocytes. Each of these cell types have been shown to synthesize IL-6 (Horowitz and Lorenzo 1996), but their presence in the tissues in this study did not have a significant relationship with IL-6 levels in synovial fluid. Although Shanbhag et al. reported IL-6 production by PE-stimulated macrophages in vitro (Shanbhag et al. 1995), others found a co-culture system with osteoblasts necessary for IL-6 synthesis (Horowitz and Gonzales 1997). Therefore, perhaps osteoblasts contribute significantly to IL-6 production following TJR.

Previous studies have shown that cytokine release is correlated with the presence of osteolysis. In particular, osteolysis around cemented arthroplasties correlated with expression of IL-1β and IL-6 mRNA expression in interface tissue shown by in situ hybridization (Goodman et al. 1998). Others measured cytokine levels in conditioned medium

following culture of periprosthetic membranes and found that IL-1β (Kim et al. 1993), (Chiba et al. 1994) and IL-6 (Chiba et al. 1994) were related to the presence of focal osteolysis.

We found no relationship between synovial fluid levels of IL-1β, IL-6, IL-8 or IL-10 with either degree of radiolucency or presence of gross loosening of the implant. This may indicate that cytokine levels in synovial fluid differ from those found locally in interface tissue. Fluid pressures in the joint space fluctuate (Hendrix et al. 1983) and many believe that synovial fluid is forced along the implant interface, particularly if there is osteolysis or errors in fixation during surgery (Schmalzried et al. 1992, Takei et al. 2000). Therefore, cells in the synovial capsule and those in the bone-implant interface may have the opportunity both to be affected by cytokine levels in the synovial fluid and also to secrete cytokines into the fluid themselves (Schmalzried et al. 1992, Nivbrant et al. 1999, Takei et al. 2000). The discrepancy between the results from our study and those already mentioned may indicate that although cytokine levels may correlate with focal osteolysis in adjacent areas, this relationship does not extend to more linear osteolysis and gross loosening. Furthermore, several clinical studies have noted that radiological appearances and, in particular, radiolucent areas bear no relationship to the clinical findings (Wroblewski and Siney 1993, Neuman et al. 1994). Owing to difficulties in achieving consistency in setting magnification, pelvic tilt, femoral rotation and flexion during x-ray, degree of radiolucency may not be a reliable indication of aseptic loosening. This may also account for the lack of correlation between clinical features and the amount of wear debris present, as the number of particles present at areas of focal osteolysis is believed to be critical (Kobayashi et al. 1997). It is interesting to note, however, that there was a relationship between migration of the implant and the presence of PE debris. PE is often considered to be the most significant type of wear debris involved in aseptic loosening (Doorn et al. 1996).

In vitro studies have shown that cytokine release by macrophages can be stimulated by addition of particulate debris in a dose- and time-dependent manner (Glant et al. 1993, Haynes et al. 1993, Maloney et al. 1996). Similarly, in our study, the amount of PE and the total amount of wear debris correlated with the expression of all 4 cytokines tested. Yet the presence of metal and 'other' types of particulates did not positively correlate with all cytokines. It is noted, however, that the majority of particulate debris is submicron (Doorn et al. 1996, Yamac et al. 1996) and therefore not discernible by light microscopy, so perhaps if these very small particles could be counted, levels of the other cytokines would indeed correlate with the presence of metal and 'other' wear. Similarly, secretion of the cytokines into synovial fluid may not be constant, therefore degradation of the cytokines following secretion into synovial fluid may occur and account for the lack of correlation with both loosening and specific types of wear debris.

Of interest is the finding that there were no significant differences in any of the histological or clinical features between hip and knee joints. We found a significantly higher IL-6 content, however, in the synovial fluid from the hip joints than in that from knees. Why IL-6, and not any of the other cytokines tested, should differ between joints is not clear; this could be related to the finding that the number of macrophages was not correlated with IL-6 production while the other cytokines were. If osteoblasts were the main producers of IL-6 in these tissues, the differences seen between joints may be related to site differences in bone cell biology. Others have shown that osteoblastic phenotype and maturation depend on skeletal origin (Pfeilschifter et al. 1993, Kasperk et al. 1995, Rawlinson et al. 1995). This may affect the production of factors such as IL-6 and account for the site-specific differences seen with this cytokine.

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Al Saffar N, Revell P, Khwaja H, Bonfield W. Assessment of the role of cytokines in bone resorption in patients with total joint replacement. J Mater Sci: Mater Med 1995; 6: 762-7.

Bresnihan B, Cunnane G, Youssef P, Yanni G, Fitzgerald O, Mulherin D. Microscopic measurement of synovial membrane inflammation in rheumatoid arthritis: Proposals for the evaluation of tissue samples by quantitative analysis. Br J Rheumatol 1998; 37 (6): 636-42.

Chiba J, Rubash H E, Kim K J, Iwaki Y. The characterization of cytokines in the interface tissue obtained from failed cementless total hip arthroplasty with and without femoral osteolysis. Clin Orthop 1994; 300: 304-12.

Doorn P, Campbell P, Amstutz H. Metal versus polyethylene wear particles in total hip replacements. Clin Orthop 1996; 329S: S206-S216.

Glant TT, Jacobs JJ, Molnar G, Shanbhag AS, Valyon M, Galante JO. Bone-resorption activity of particulate-stimulated macrophages. J Bone Miner Res 1993; 8 (9): 1071-9.

Goodman S B, Huie P, Song Y, Schurman D, Maloney W, Woolson S, Sibley R. Cellular profile and cytokine production at prosthetic interfaces - Study of tissues retrieved from revised hip and knee replacements. J Bone Joint Surg (Br) 1998; 80 (3): 531-9.

Harris W H, McCarthy J C, O'Neill D A. Femoral component loosening using contemporary techniques of femoral cement fixation. J Bone Joint Surg (Am) 1982; 64 (7): 1063-7.

Haynes D, Rogers S, Hay S, Pearcy M, Howie D. The differences in toxicity and release of bone-resorbing mediators induced by titanium and cobalt-chromium-alloy wear particles. J Bone Joint Surg (Am) 1993; 75 (6): 825-34.

Hendrix R, Wixson R, Rana N, Rogers L. Arthrography after total hip arthroplasty: a modified technique used in the diagnosis of pain. Radiology 1983; 148: 647-52.

Horowitz M, Lorenzo J. Principles of bone biology. San Diego, Academic Press 1996.

Horowitz S, Gonzales J. Effects of polyethylene on macrophages. J Orthop Res 1997; 15: 50-6.

Hsu J, Bukata S, Pollice P, Puzas J, Reynolds P, Rosier R, Hicks D, Stroyer B, O'Keefe R. Paracrine induction of interleukin-10 in the pathologic periprosthetic bone resorption of aseptic loosening. J Bone Miner Res (Suppl) 1997; 12: S341.

Hutton C W, Hinton C, Dieppe P A. Intraarticular variation of synovial changes in knee arthritis– biopsy study comparing changes in patellofemoral synovium and the medial tibiofemoral synovium. Br J Rheumatol 1987; 26 (1): 5-8.

Ishiguro N, Kojima T, Ito T, Saga S, Anma H, Kurokouchi K, Iwahori Y, Iwase T, Iwata H. Macrophage activation and migration in interface tissue around loosening total hip arthroplasty components. J Biomed Mater Res 1997; 35: 399-406.

Jasty M, Goldring S, Harris W. Comparison of bone cement membrane around rigidly fixed versus loose total hip implants. Trans Orthop Res Soc 1984; 9: 125.

Kahle P, Saal J G, Schaudt K, Zacher J, Fritz P, Pawelec G. Determination of cytokines in synovial fluids – correlation with diagnosis and histomorphological characteristics of synovial tissue. Ann Rheum Dis 1992; 51 (6): 731-4.

Kasperk C, Wergedal J, Strong D, Farley J, Wangerin K, Gropp H, Ziegler R, Baylink D J. Human bone cell phenotypes differ depending on their skeletal site of origin. J Clin Endocrin Metab 1995; 80 (8): 2511-7.

Kim K J, Rubash H E, Wilson S C, Dantonio J A, McClain E J. A histologic and biochemical comparison of the interface tissues in cementless and cemented hip prostheses. Clin Orthop 1993; 287: 142-52.

Kobayashi A, Freeman M A R, Bonfield W, Kadoya Y, Yamac T, Al Saffar N, Scott G, Revell P A. Number of polyethylene particles and osteolysis in total joint replacements. A quantitative study using a tissue-digestion method. J Bone Joint Surg (Br) 1997; 79 (5): 844-8.

Lassus J, Waris V, Xu J, Li T, Hao J, Nietosvaara Y, Santavirta S, Konttinen Y. Increased interleukin-8 (IL-8) expression is related to aseptic loosening of total hip replacement. Arch Orthop Trauma Surg 2000; 120 (5-6): 328-32.

Maloney W, James R, Smith R. Human macrophage response to retrieved titanium alloy particles in vitro. Clin Orthop 1996; 322: 268-78.

Murray D, Rushton N. Macrophages stimulate bone resorption when they phagocytose particles. J Bone Joint Surg (Br) 1990; 72: 988-92.

Neuman L, Freund K, Sorenson K. Long-term results of Charnley total hip replacements. Review of 92 patients at 15 to 20 years. J Bone Joint Surg (Br) 1994; 76 (2): 245-51.

Nivbrant B, Karlsson K, Kärrholm J. Cytokine levels in synovial fluid from hips with well-functioning or loose prostheses. J Bone Joint Surg (Br) 1999; 81 (1): 163-6.

Pfeilschifter J, Diel I, Pilz U, Brunotte K, Naumann A, Ziegler R. Mitogenic responsiveness of human bone cells in vitro to hormones and growth factors decreases with age. J Bone Miner Res 1993; 8 (6): 707-17.

Rae T. A study on the effects of particulate metals of orthopaedic interest on murine macrophages in vitro. J Bone Joint Surg (Br) 1975; 57 (4): 444-50.

Rawlinson S C F, Mosley J R, Suswillo R F L, Pitsillides A A, Lanyon L E. Calvarial and limb bone cells in organ and monolayer culture do not show the same early responses to dynamic-mechanical strain. J Bone Miner Res 1995; 10 (8): 1225-32.

Rooney M, Condell D, Quinlan W, Daly L, Whelan A, Feighery C, Bresnihan B. Analysis of the histologic variation of synovitis in rheumatoid arthritis. Arthritis Rheum 1988; 31 (8): 956-63.

Schmalzried T, Jasty M, Harris W. Periprosthetic bone loss in total hip arthroplasty. Polyethylene wear debris and the concept of the effective joint space. J Bone Joint Surg (Am) 1992; 74 (6): 849-63.

Shanbhag A, Jacobs J, Black J, Galante J, Glant T. Macrophage/particle interactions: Effect of size, composition and surface area. J Biomed Mater Res 1994; 28: 81-90.

Shanbhag A, Jacobs J, Black J, Galante J, Glant T. Human monocyte response to particulate biomaterials generated in vivo and in vitro. J Orthop Res 1995; 13: 792-801.

Takei I, Takagi M, Ida H, Ogino T, Santavirta S, Konttinen Y. High macrophage-colony stimulating factor levels in synovial fluid of loose artificial hip joints. J Rheumatol 2000; 27 (4): 894-9.

Wroblewski B, Siney B. Charnley low-friction arthroplasty of the hip. Long-term results. Clin Orthop 1993; 292: 191-201.

Xu J, Konttinen Y, Lassus J, Natah S, Ceponis A, Solovieva S, Aspenberg P, Santavirta S. Tumor necrosis factor-alpha (TNF-α) in loosening of total hip replacement (THR). Clin Exp Rheumatol 1996; 14: 643-8.

Xu J, Konttinen Y, Waris V, Patiala H, Sorsa T, Santavirta S. Macrophage-colony stimulating factor (M-CSF) is increased in the synovial-like membrane of the periprosthetic tissues in the aseptic loosening of total hip replacement (THR). Clin Rheumatol 1997; 16: 243-8.

Yamac T, Kobayashi A, Bonfield W, Kadoya Y, Freeman M, Scott G, Revell P. Extraction and characterisation of metallic wear particles in total joint replacements. Fifth World Biomaterials Congress 1996; II: 861.