

Metabolic Activity of Osteoblasts from Periprosthetic Trabecular Bone in Failed Total Hip Arthroplasties and Osteoarthritis as Markers of Osteolysis and Loosening

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ABSTRACT. Objective. This study explored whether osteoblast metabolism in trabecular bone of failed total hip replacement (fTHR) in primary osteoarthritis (OA) plays a role in differential failure.

Methods. Osteoblast cell cultures were prepared from metaphyseal trabecular bone of normal individuals, OA patients, OA patients with fTHR with massive cavitory osteolysis (fTHR-O) and without massive cavitory osteolysis (fTHR-NO). Osteoblasts were characterized by measuring osteocalcin, cellular alkaline phosphatase (ALP), and urokinase plasminogen activator (uPA) activity. The cellular metabolic activity was also evaluated by measuring the production of interleukin (IL)-1 β , IL-6, and prostaglandin E₂ (PGE₂).

Results. ALP activity was increased in osteoblasts from patients with OA and fTHR-O compared to normal controls and fTHR-NO. Osteocalcin release was increased only in fTHR-O compared to all other groups. uPA activity was highest in the subgroup of "high metabolic OA" and in fTHR-O, while it was lowest in the subgroup of "low metabolic OA" and in fTHR-NO. IL-6 and PGE₂ production was higher in the high metabolic OA and fTHR-O compared to fTHR-NO and low metabolic OA patients.

Conclusion. Osteoblasts from fTHR-O and a subgroup of OA patients present similarly increased osteoblast markers compared to normal subjects, the low metabolic OA subgroup, and fTHR-NO. This information suggests the differential role of osteoblasts in osteolysis pathophysiology in primary THR surgery. The pertinence of the differential high and low metabolic activities of osteoblasts to the pathophysiology of OA remains to be fully established. (J Rheumatol 2002;29:1437–45)

Key Indexing Terms:

OSTEOBLASTS
OSTEOLYSIS

METABOLIC MARKERS

TOTAL HIP REPLACEMENT
OSTEOARTHRITIS

Total hip replacement (THR) is the surgical procedure of choice for patients over the age of 60 suffering from osteoarthritis (OA) of the hip. About 240,000 THR are performed every year in the USA and Canada, and roughly 10% of these surgeries are revisions due to aseptic loosening. The pathophysiology of osteolysis in failed THR (fTHR) is related to a heavy macrophage response to

implant debris at the bone-implant interface and the expression of several cytokines, prostaglandins, metalloproteases, and nitric oxide. Osteoblast activity in this pathology is still underestimated, even though these cells are known to respond to debris¹. It has been demonstrated that macrophages differentiate directly into osteoclastic bone resorbing cells², and the main pathway to macrophage differentiation into osteoclasts responsible for bone resorption in fTHR is osteoblast dependent³. Thus, the analysis of osteoblastic metabolism in this process may contribute by discriminating the different degrees of osteolysis (cavitory or linear) and could be used as an early marker in THR failure.

Any given series of THR patients whose clinical variables are comparable (same basic pathology, surgeon, implant, exposure to identical debris, and similar age and physical activity) present subsets of patients with osteolysis ranging from very mild to massively catastrophic. Most OA patients also develop some osteolytic lesions (bone cysts) over time. Recent data showed that 2 subgroups of OA can be discriminated based upon high metabolic and low metabolic subchondral osteoblast activities⁴. Interestingly,

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human osteoblasts can also be discriminated into functionally different populations via intercellular adhesion molecule (ICAM) expression⁵. Thus, it is reasonable to assume that some patients with OA may have a much stronger and individual response to wear debris due to an unbalanced osteoblastic status.

Radiographic changes are slow in onset and become evident only after extensive resorption. Dual energy x-ray absorptiometry (DEXA) is the gold standard in assessing bone mass, but it is limited by a static measurement that does not offer any information on the current rate of bone turnover⁶. Resorption and formation are usually coupled, but changes in formation markers lag several months behind changes in resorption markers⁷. A biochemical marker would be ideal for noninvasive diagnosis and assessment of treatment prior to any radiographic evidence of significant bone loss. In addition, if a biological marker based upon cellular metabolism could be used in selected patients to predict the likelihood of massive osteolysis, then it may also theoretically be used as early as the time of primary total hip replacement.

The objective of this study was to investigate potential early markers of osteolysis in OA patients that have predictive value for failed THR. We assessed the metabolic functions of osteoblasts from trabecular bone of OA patients and patients with fTHR with and without massive osteolysis.

MATERIALS AND METHODS

Patient characteristics. Normal control specimens were obtained from hip fracture specimens of 10 patients (71 ± 8 yrs old, mean ± SD, 6 female/4 male) with no history of metabolic bone disease or clinical record of OA and no macroscopic evidence of OA at surgery, to permit comparisons with fTHR and OA patients.

Osteoporosis was an exclusion criterion in this group. None of the 10 patients had a history of osteoporotic fracture. Also excluded were those with available bone mineral density (BMD) results at time of surgery of 2.5 standard deviations below the mean for a young adult. If no BMD at surgery was available, we applied the SCORE (Simple Calculated Osteoporosis Risk Estimation) questionnaire in all eligible patients prior to enrollment^{8,9}. The SCORE questionnaire is a tool to assist physicians in identifying women who might require bone densitometry. SCORE results are compared to hip and lumbar spine bone density assessed by DEXA. In women aged 60–70 years old, a SCORE cut point of 8 displays a sensitivity of 0.90, 95% CI (0.80, 0.97) and a specificity of 0.20, 95% CI (0.11, 0.29). Older women require higher SCORE cut points. The use of SCORE as an initial measure for identifying those at risk for osteoporosis limited unnecessary tests in patients seen in the emergency room and waiting for surgery. Moreover, none of these patients were taking calcium, vitamin D, hormone replacement therapy, or bisphosphonates prior to surgery. Based on these criteria, out of 150 potential candidates, we retained the present 10 patients who had no history of type I, II, or III osteoporosis.

OA specimens were obtained from 12 patients (68 ± 11 yrs, 5 female/2 male) undergoing THR surgery for OA based on the American College of Rheumatology criteria¹⁰. These specimens were divided in 2 groups, low metabolic OA (n = 7) and high metabolic OA (n = 5), based on whether individual results for metabolic markers were at least 2 SD higher or lower than those of the normal group. No attempt was made to correlate these values to the clinical presentation of hip OA.

Two distinct groups of revised implants were identified: 6 patients

without massive cavitory osteolysis (fTHR-NO, Table 1) and 6 patients with loose implants with massive cavitory osteolysis (fTHR-O, Table 1). The mean age (61.7 ± 5.6 yrs, 4 female/2 male and 65.4 ± 5.3 yrs, 4 female/2 male, respectively) and the time of revision (57.0 ± 12.9 mo and 49.2 ± 11.5 mo, respectively) were similar for both groups. All fTHR patients were diagnosed as having primary OA at the time of surgery¹⁰. Only failed hybrid implants with a loose stem were analyzed in this study to provide for standardization of the data. The indication for a revision included pain and/or evidence of clinically significant loosening or mechanical failure of the implant. Loosening was confirmed by the presence of radiolucent lines at the bone interface, a fracture around the stem, and/or the migration of a component. The radiographs from the loose implants with cavitory osteolysis (fTHR-O) contained at least one area showing ballooning and radiolucent zones with scalloped edges adjacent to the cement mantle or bone (Figure 1)¹¹. fTHR-NO was defined as the absence of osteolysis or the presence of a linear radiolucent line at the bone-cement interface.

Our study was approved by the Institutional Research Committee/Institutional Ethics Committee and all patients signed an informed consent.

Trabecular bone sample selection. Trabecular bone specimens were prepared from plug explants taken from Gruen zones I and/or VII of the metaphyseal region of the proximal femur to provide for standardization of sampling. Bone specimens from fTHR-O were prepared from areas immediately adjacent to osteolytic lesions, and zones corresponding to periprosthetic bone were separated in fTHR-NO. They were divided by microscopic examination (dissection microscope, Bausch and Lomb, Rochester, NY, USA) to ensure all remains of interface membranes had been discarded¹². Any repair or mesenchymal tissues were eliminated whenever present and no cortical bone was included.

In vitro osteoblast cell cultures. Osteoblast cell cultures were prepared using published methods^{13,14}. These cells retain their *in vivo* metabolic characteristics in *in vitro* cultures and altered metabolism can be distin-

Table 1. Patient characteristics in failed total hip replacement, with and without cavitory osteolysis. Two distinct groups of revised implants were identified: 6 patients without cavitory osteolysis (fTHR-NO) and 6 patients with loose implants with massive cavitory osteolysis (fTHR-O). The mean age (61.7 ± 5.6 years, 4 F/2M and 65.4 ± 5.3, 4 F/2M, respectively) and the time of revision (57.0 ± 12.9 mo and 49.2 ± 11.5 mo, respectively) were similar for both groups. All fTHR patients were diagnosed as having primary OA at the time of surgery. Only failed hybrid implants with a loose stem were analyzed in this study to provide for standardization of the data.

Patient	Age/Sex	Mo to Revision	Degree of Wear	Cause of Failure
1	75 F	34	Moderate	Linear/cavitory osteolysis
2	79 F	86	Moderate	Cavitory osteolysis
3	77 M	34	Massive	Linear/cavitory osteolysis
4	68 F	40	Massive	Femoral fracture/cavitory osteolysis
5	63 F	64	Moderate	Linear/cavitory osteolysis
6	59 M	49	Massive	Cavitory osteolysis
7	71 F	34	Massive	Hip dislocation/linear osteolysis
8	78 F	118	Massive	Linear osteolysis/insert dislocation
9	68 M	62	Massive	Linear osteolysis
10	42 F	38	Moderate	No osteolysis/periprosthetic fracture
11	62 M	49	Moderate	No osteolysis/periprosthetic fracture
12	59 F	41	Mild/moderate	Massive insert wear/linear osteolysis

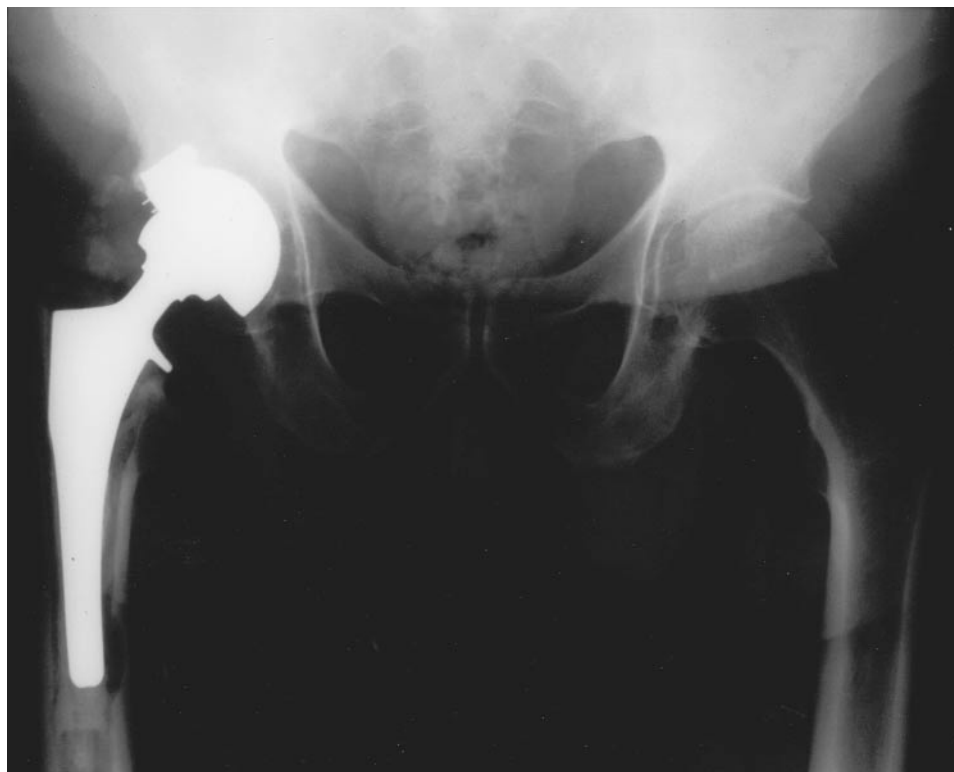


Figure 1. Radiographic appearance of a massive cavitory osteolytic lesion in a failed total hip replacement. Note the expansive lesion that extends beyond the tip of the stem. Moreover, there is also extensive bone resorption in the metaphyseal region of the proximal femur.

guished *in vitro* under these conditions as reported^{5,13-15}. First passage cells only were used in these experiments. Briefly, bone samples were cut into small pieces (2 mm²) prior to their sequential digestion (3 times) with collagenase (*Clostridium histolyticum*, Type I; Sigma, St. Louis, MO, USA) at 1 mg/ml, trypsin at 0.5 mg/ml in a BGJb media without serum (Sigma) and containing 2 mM EDTA to promote extracellular calcium chelation. The digestions were performed at 37°C twice for 20 min and once for 4 h. This removed both adherent and remaining bone marrow cells from the bone pieces. After washing with the same medium, the digested bone pieces were put into 25 cm² plastic culture dishes (Falcon, Lincoln Park, NJ, USA) and covered with BGJb medium containing 20% fetal bovine serum (FBS) (Wisent, St. Bruno, Quebec, Canada) and 1% penicillin/streptomycin and 50 µg/ml ascorbic acid. This medium was changed every 2 days until cell outgrowths appeared around the explants (usually 7–10 days after initial collagenase digestion). At this stage, the medium was changed to 10% FBS until cells reached confluency (28–35 days). These cells were split once at high density (50,000 cells/cm²) and put into 24 well plates. After 2 days of recovery, the cells were fed once with the same medium and 2 days later with a serum-free media consisting of Ham's F12/Dulbecco's modified Eagle's medium (DMEM; Sigma) containing 50 µg/ml ascorbic acid and 0.1% bovine serum albumin.

Conditioning was performed for the last 2 days of culture, in the presence or absence of 50 nM 1,25 (1,25(OH)₂D₃) in Ham's F-12/DMEM containing 2% charcoal stripped FBS, which yields maximal stimulation of alkaline phosphatase (ALP) activity and osteocalcin secretion. The medium was collected at the end of the incubation and frozen at -80°C prior to assay. Cells were washed twice with phosphate buffered saline (PBS), pH 7.4 and solubilized in ALP buffer (100 mM glycine, 1 mM MgCl₂, 1 mM ZnCl₂, 1% Triton X-100; pH 10.5) for 60 min with agitation at 4°C. Human bone cells obtained under these culture conditions show an osteoblast-like phenotype¹³⁻¹⁵.

Determination of osteocalcin and cellular ALP. Osteocalcin release was measured in conditioned Ham's F-12/DMEM media prepared for the last 2 days of culture of osteoblasts. Nascent osteocalcin was determined by a specific enzyme immunoassay (Biomedical Technologies, Inc., Stoughton, MA, USA). The detection limit of this assay is 0.5 ng/ml, and 2% charcoal treated FBS contains less than 0.1 ng/ml osteocalcin. Cellular ALP activity was determined as the release of p-nitrophenol hydrolyzed from p-nitrophenyl phosphate (12.5 mM final concentration) at 37°C for 30 min after solubilizing the cells in ALP buffer as described above. Protein determination was performed by the bicinchoninic acid method¹⁶. Determination of urokinase plasminogen activator activity, prostaglandin E₂ abundance and cytokine (IL-1β, IL-6) levels in cell cultures.

Determination of urokinase plasminogen activator activity. Conditioned media from confluent osteoblasts fed with Ham's F-12/DMEM media without FBS, but containing 1% insulin-transferrin-selenium mix (ITS) (Sigma) for their last 2 days of culture, were used for this determination. We employed the procedure of LePrince, *et al*¹⁷ to determine the activity of urokinase plasminogen activator (uPA) via the hydrolysis of the specific substrate DL-Val-Leu-Arg-p-nitroanilide (Sigma), which releases p-nitroaniline detectable at 405 nm. Internal controls were performed with media alone that contained 1% ITS, and their values were subtracted from those of the samples.

Determination of prostaglandin E₂ (PGE₂). The amount of PGE₂ released into the medium was assessed using a PGE₂ enzyme immunoassay kit from Cayman Chemical (Ann Arbor, MI, USA). This assay is based on the competition between PGE₂ and a PGE₂-acetylcholinesterase conjugate (PGE₂ tracer) for a limiting amount of PGE₂ monoclonal antibody. The sensitivity was 9 pg/ml, and the working range was between 10 and 1000 pg/ml, based on a logarithmic transformation. The majority of PGE₂ synthesized by osteoblasts is immediately released into the medium rather

than stored in the cell layer¹⁸. For this reason, no examination of the PGE₂ content of the cell layer was done.

IL-1 β and IL-6 ELISA assays. The levels of IL-1 β and IL-6 were determined in the culture medium using specific solid phase, double antibody ELISA. A high sensitivity ELISA kit was used to measure IL-1 β . The IL-1 β kit (high sensitivity kit specific for mature IL-1 β) was from Cistrion Biotechnology (Pine Brook, NJ, USA) and IL-6 from R&D Systems (Minneapolis, MN, USA). Sensitivities of the assays in the ELISA plates were IL-1 β : 0.3 pg/ml and IL-6: 3 pg/ml. Each ELISA was performed in duplicate according to the manufacturer's specifications. The reaction was measured on a micro-ELISA Vmax photometer (Molecular Devices Corp., Menlo Park, CA, USA).

Statistical analysis. Results are expressed as the mean \pm SEM. Assays were performed in triplicate except where indicated. Statistical analysis was performed by 2 tailed Mann-Whitney U test. P values < 0.05 were considered significant.

RESULTS

ALP activity and osteocalcin levels. After bacterial collagenase digestion, cells began to migrate out of the explants after 7–14 days of culture. After 4–5 weeks of culture, cell cultures became confluent. Cell growth was similar for osteoblasts from failed total hip replacement (fTHR), OA, and normal patients. Primary bone cell cultures from normal, OA, and fTHR all showed an approximate 2-fold increase in ALP upon stimulation with 50 nM 1,25(OH)₂D₃. ALP levels observed in normal cells were low and within the expected range^{4,15}. These levels were significantly higher in OA and fTHR with massive cavitory osteolysis (fTHR-O) osteoblast-like cells compared with normal cells under 1,25(OH)₂D₃ stimulated conditions (Figure 2). Osteoblasts from fTHR without massive cavitory osteolysis (fTHR-NO) showed an ALP activity similar to normal.

The measurement of osteocalcin under basal conditions revealed no statistically significant difference in OA and fTHR-O osteoblast-like cells compared to normal (Figure 3). The only significant difference in osteocalcin production between normal, OA, and fTHR cells under 1,25(OH)₂D₃ stimulation was found in the fTHR-O group. Biomarkers were not randomly distributed within the cell cultures *in vitro* but showed more than 99% uniform expression throughout cells (data not shown).

Determination of uPA activity and IL-1 β , IL-6, and PGE₂ levels. The highest uPA activity was found in the culture medium of osteoblasts from the high metabolic OA and fTHR-O groups (Figure 4). Levels in the high metabolic OA and fTHR-O groups were about 3–4 fold higher than in normal cells. Osteoblasts from fTHR-NO showed uPA activity to be lower than normal.

IL-1 β production was found to be higher only in the fTHR-O group compared to normal (Figure 5A). There was a significant increase in IL-6 levels in high metabolic OA and fTHR-O cells when compared to normal and fTHR-NO (Figure 5B). PGE₂ levels were significantly increased over normal in the high metabolic OA and fTHR-O groups (Figure 6). In contrast, the PGE₂ level in the fTHR-NO group was slightly lower compared to the normal group and was significantly lower than that found in the fTHR-O group. No significant difference was observed in PGE₂ levels between the high metabolic OA and fTHR-O groups.

DISCUSSION

Aseptic loosening has remained the primary obstacle

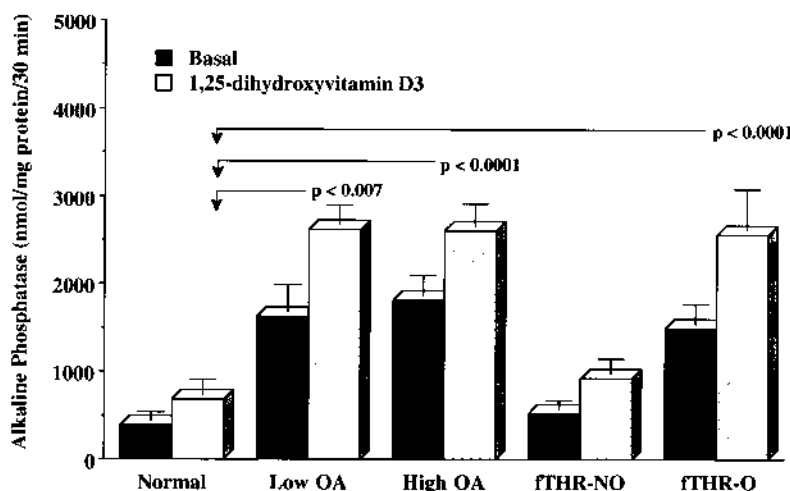


Figure 2. Cellular levels of alkaline phosphatase (ALP) activity in osteoblast cells from normal (n = 10), low metabolic OA (n = 7), high metabolic OA (n = 5), failed total hip replacement with massive cavitory osteolysis (fTHR-O) (n = 6), and without osteolysis (fTHR-NO) (n = 6) under basal conditions and 50 nM 1,25(OH)₂D₃ stimulation. ALP activity was determined in quadruplicate dishes per experiment, using *p*-nitrophenyl phosphate (see Materials and Methods). Results indicate significant increases in ALP activities in osteoblasts from fTHR-O under 1,25(OH)₂D₃ stimulation ($p < 0.0001$) and OA osteoblasts (high metabolic OA, $p < 0.0001$; low metabolic OA, $p < 0.007$) compared to normal cells. Osteoblasts from fTHR-NO show ALP activity similar to normal. Values represent the mean \pm SEM. Statistical analysis was performed by 2 tailed Mann-Whitney U test.

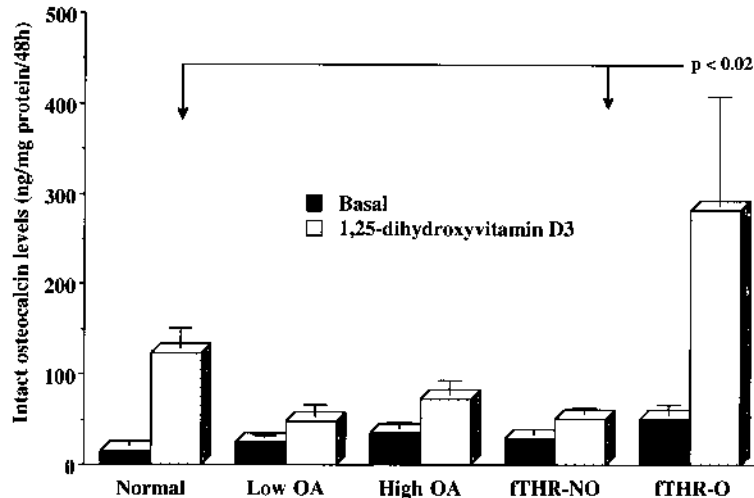


Figure 3. Secretion of intact osteocalcin by human osteoblast cell cultures under basal and 1,25(OH)₂D₃ stimulation. The culture medium (quadruplicates) was used for determination of osteocalcin release. Osteocalcin was determined by enzyme immunoassay (see Materials and Methods). The level of osteocalcin in 1,25(OH)₂D₃ stimulated osteoblasts from fTHR-O (n = 6) was found to be significantly higher (p < 0.02) than that found in normal cells and fTHR-NO. Values shown represent the mean ± SEM. Statistical analysis was performed by 2 tailed Mann-Whitney test.

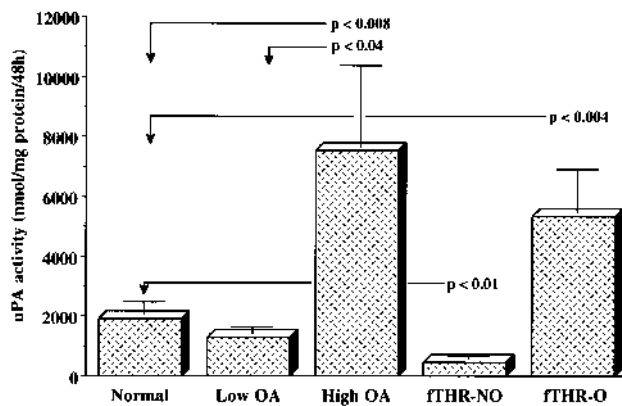


Figure 4. Urokinase plasminogen activator (uPA) activity in culture medium of osteoblast cells. Cells from trabecular bone of normal (n = 10), low metabolic OA (n = 7), high metabolic OA (n = 5), fTHR-O (n = 6), and fTHR-NO (n = 6) patients were incubated as described in Materials and Methods. Levels of uPA were determined by detecting the release of p-nitroaniline at 405 nM via the hydrolysis of a specific peptide substrate. The uPA activity was found to be significantly higher in the high metabolic OA (p < 0.008 and p < 0.04) and fTHR-O (p < 0.004) groups compared to normal, and higher in the high metabolic OA versus low (P < 0.04) metabolic OA groups. Osteoblasts from fTHR-NO show uPA activity lower than normal (p < 0.01). Values shown represent the mean ± SEM and statistical analysis was performed by 2 tailed Mann-Whitney U test.

limiting the longevity of total hip arthroplasties. Our results indicate a peculiar osteoblastic metabolism that could be used as a biologic marker of osteolysis observed in failed THR. Osteoblasts are the lining cells in trabecular bone and are essential for osteoclast recruitment and activation/modulation of bone resorption via cell-cell and chemical signals⁵.

Recent data have shown that at least 2 subpopulations of osteoblasts exist in OA trabecular bone: one that induces osteoclastogenesis, while the other is involved in bone matrix formation; moreover, cell adhesion of osteoblasts is biased toward inducing osteoclastogenesis rather than bone formation⁵. Moreover, our laboratory has already described an altered metabolism in osteoblasts from subchondral and trabecular bone in OA^{4,15} in failed THR with cavitory osteolysis and without cavitory osteolysis¹⁹. Our previous results suggest that we have in fact 2 different subpopulations of osteoblasts in OA and fTHR.

Normal osteoblasts in the present study were obtained from patients with hip fractures, and this may raise the possibility that, since 6 out of 10 patients were postmenopausal women, a mixed response may be obtained *in vitro* due to osteoporosis in some of these normal patients. However, a number of studies have indicated that, although osteoporotic patients have altered bone metabolic capacities, this may not be due to altered osteoblastic capacities per se. Indeed, Stenderup, *et al* concluded that the number and proliferative capacity of osteoprogenitor cells are maintained during aging and in patients with osteoporosis²⁰. Likewise, although the dose dependent increase of osteocalcin production by osteoblasts *in vitro* following stimulation with 1,25(OH)₂D₃ is reduced in older individuals, it does not seem to vary widely with gender²¹ nor with osteoporosis^{22,23}, which would indicate that our normal group is fairly representative of an aged matched control for the OA or FHR group of individuals. Lastly, whether these individuals were taking calcium, osteotropic hormones, or bisphosphonates prior to surgery may not adversely affect the

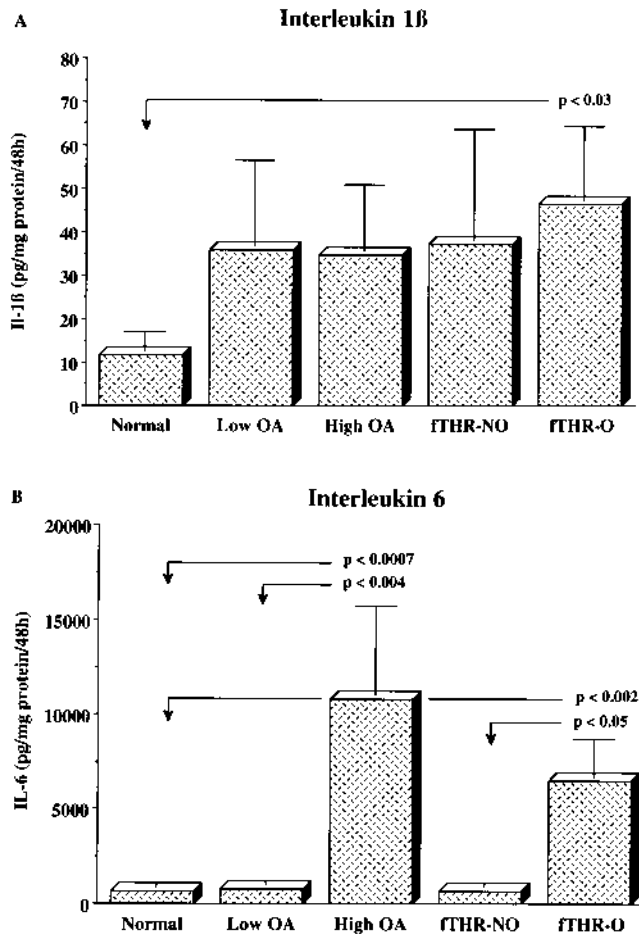


Figure 5. Levels of interleukin-1 β (IL-1 β) (A) and interleukin-6 (IL-6) (B) in the culture medium of osteoblast cells. Normal (n = 10), low metabolic OA (n = 7), high metabolic OA (n = 5), fTHR-O (n = 6) and fTHR-NO (n = 6) osteoblast cells were grown as described in Materials and Methods. The levels of IL-1 β and IL-6 were determined in the culture medium using specific solid phase, double antibody ELISA. Each assay was performed in duplicate. Cells from fTHR-O had a significant increase in IL-1 β levels compared to normal ($p < 0.03$). There was a significant increase in IL-6 levels in the high metabolic OA subgroup ($p < 0.0007$) and fTHR-O ($p < 0.002$) when compared to normal. The levels of IL-6 were also higher in high metabolic OA ($p < 0.004$) compared to low metabolic OA and in fTHR-O ($p < 0.05$) compared to fTHR-NO. Values are expressed as the mean \pm SEM; statistical analysis was done by 2 tailed Mann-Whitney U test.

outcome of this *in vitro* study. First, given the longterm culture used, the *in vivo* effect of calcium or osteotropic hormones would be very small if not absent; and second, our own *in vitro* studies with bisphosphonates (unpublished data) and reports from other laboratories²⁴ indicate no effect on osteoblast viability, proliferation, ALP activity, osteocalcin secretion, or mineral deposition.

Total joint arthroplasties are anchored to bone. The majority of patients undergoing total joint replacements are OA patients. This begs the question of whether the bony bed surrounding these implants might take a more active part in

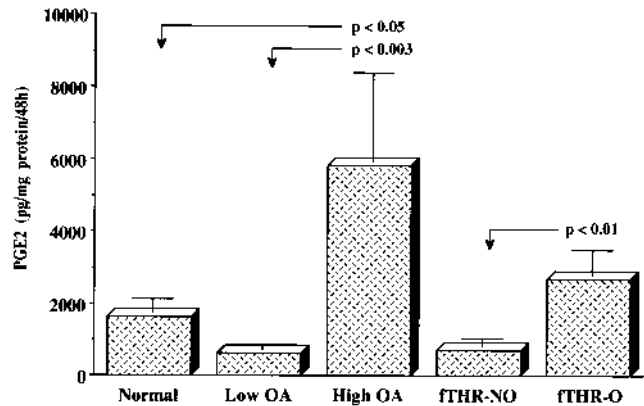


Figure 6. Level of prostaglandin E₂ (PGE₂) in the culture medium of osteoblast cells. Normal (n = 10), low metabolic OA (n = 7), high metabolic OA (n = 5), fTHR-O (n = 6), and fTHR-NO (n = 6) osteoblast cells were grown to confluence as described in Materials and Methods. The amount of PGE₂ was assessed in the medium by use of a PGE₂ enzyme immunoassay kit. Assays were performed in duplicate. PGE₂ levels in high metabolic OA were slightly increased over those found in normal cells ($p < 0.05$) and low metabolic OA ($p < 0.003$). Moreover, PGE₂ levels found in the fTHR-O group were increased over those found in fTHR-NO cells ($p < 0.01$). Values are expressed as mean \pm SEM; statistical analysis was performed by 2 tailed Mann-Whitney U test.

the pathophysiology of implant loosening. Classically, most of the changes in loosening are thought to come from wear debris and macrophage reaction. This cascade has already been well described¹. Nevertheless, only 10–30% of all patients with the same type of implant will develop osteolysis and loosening, even though they are all theoretically exposed to similar volume, type, and size of wear debris. This rather idiosyncratic response was thought to be part of an immune response to this debris; however, the presence of nonactivated resting cells suggests a rather modest immune response in aseptic loosening and osteolysis²⁵.

The increased osteocalcin release and ALP activity observed in the fTHR-O group may indicate enhanced metabolic activity resembling the recently described ICAM-1+ osteoblasts which could lead to abnormal bone formation *in vivo*⁵. It would also reflect acquired traits/features due to wear debris. Indeed, adaptive bone remodeling adjacent to an orthopedic implant may reflect alterations in the mechanical environment of bone engendered by implantation of the prosthesis²⁶, whereas nonadaptive changes, such as those associated with wear debris, are often described by the term osteolysis. Previous studies have shown that ultra-high molecular weight polyethylene particles have no effect on osteocalcin production by a human osteoblast-like cell line, MG63, *in vitro*²⁷, whereas a decrease in ALP is observed in these cells and appears to be a selective pathway altered in response to the particles. However, although exposed to similar wear debris, fTHR-O and fTHR-NO showed different osteocalcin release and ALP activity, which suggests that the debris per se could not be responsible for

this behavior in these cells. Moreover, if the results obtained in MG63 cells reflect a genuine response of osteoblasts to wear debris²⁷, osteocalcin should have been normal in fTHR-O and ALP should have been lower than in normal osteoblasts, which is not the case. This would indicate that fTHR-O osteoblasts have unique features, similar to high metabolic OA osteoblasts that may contribute to enhanced osteolysis. Both osteocalcin and ALP are markers of increased osteoblastic differentiation and activity but not necessarily mineralization of the matrix¹⁵. This suggests that an imbalance in this metabolic pathway may prevail in fTHR-O but not in fTHR-NO, which further indicates an altered metabolism of fTHR-O osteoblasts.

Increased plasminogen activation has already been identified in loosening of total hip prosthesis²⁸, and our study shows that this system is upregulated in osteoblasts, a situation that can explain local enhancement of bone turnover. The higher uPA activity level found in the high metabolic OA and fTHR-O groups suggests higher bone turnover and possible osteoclastic activity stimulation compared to fTHR-NO, normal, and OA groups^{1,5}. In normal bone turnover, bone resorption is followed by bone formation through local coupling of the osteoclast and the osteoblast. In inflammation, this coupling mechanism is disrupted with excessive bone resorption and/or diminished compensatory bone formation²⁹⁻³¹. The production of ALP and osteocalcin in response to hormonal challenge, and uPA and cytokines (IL-1 β and IL-6) has already been described as aberrant in human OA subchondral osteoblasts when compared to normal subchondral osteoblasts^{4,15}. This suggests that OA osteoblasts have an altered metabolism that may contribute to the onset and/or progression of this disease.

A combined increase in the basal levels of IL-1 β and IL-6 can account for a much higher osteoclastic response when cells are exposed to a focal stimulation. Indeed, polymethylmethacrylate powder produced a net inhibition of cell proliferation and collagen synthesis in human osteoblasts³² while enhancing osteocalcin and IL-6 production. A similar situation could then reduce periprosthetic bone formation *in vivo* by reducing osteoblast proliferation and collagen synthesis. Moreover, the increased levels of osteocalcin and IL-6 synthesis in fTHR-O osteoblasts, as shown in this study, suggest an osteoblast mediated activation of osteoclastic bone resorption. Whether this still reflects a direct effect of wear particles on osteoblasts is unlikely as cells are kept in culture for about 5 to 6 weeks before evaluating these variables. Osteocalcin has been demonstrated to have a role in osteoclast recruitment to bone surfaces³², and IL-6 is known to induce osteoclastogenesis and to directly stimulate bone resorption^{32,33}. Osteoclastogenesis may arise from the stimulation of resident macrophages found in response to biomaterial wear particles in fTHR-O by local chemical effectors produced by osteoblasts^{2,34}. Moreover, macrophages isolated from interface pseudomembrane are capable

of differentiating into osteoclasts through osteoprotegerin ligand mediated processes as osteoprotegerin inhibits this osteoblast-macrophage cell-to-cell process³⁴. Hence, in the presence of osteoblasts that express higher than normal levels of IL-6 and osteocalcin, as observed for fTHR-O, one would expect that the osteoclast recruitment and differentiation would be greater than normal in this group. In fTHR-O, there is consensus that bone resorption by osteoclasts is increased, while bone formation by osteoblasts is decreased^{27,35}. However, a comprehensive overview of this process has to account not only for bone resorption by osteoclasts, but also for the fact that osteoblasts are known to both synthesize and participate in the degradation of the bone matrix and are also capable of phagocytosis of particles like collagen fibers and small titanium particles³⁶. Hence, the question becomes, is wear debris directly or indirectly eliciting this increased osteoblast activity via chemical factors locally produced by osteoblasts? In this respect, it is noteworthy that an inverse relationship between net bone formation and particle concentration is observed²⁷. Hence, osteoblasts play a role in osteolysis by producing local factors such as macrophage-colony stimulating factor and IL-6, which induces macrophage differentiation into active osteoclasts³³.

The lower PGE₂ level in the low metabolic OA and fTHR-NO groups compared to the high metabolic OA and fTHR-O groups may have some interesting implications in the development of osteolysis in the latter 2 conditions. Indeed, drug suppression of PGE₂ synthesis by nonsteroidal antiinflammatory drugs does not suppress pseudomembrane induced bone resorption³⁷. On the other hand, the amount of PGE₂ constitutively produced by the osteoblasts is usually very low in culture and is unaffected by 1,25(OH)₂D₃ but increases when the cells are challenged with ultra-high molecular weight polyethylene particles²⁷. PGE₂ has a biphasic effect on bone formation¹⁸, since low levels are associated with increased differentiation of osteogenic cells, while increased PGE₂ production can also enhance osteoclast activity. Our results indicate that low metabolic OA and fTHR-NO osteoblasts produce lower levels of PGE₂ than fTHR-O and high metabolic OA osteoblasts *in vitro*. This could theoretically account for a higher differentiation of osteogenic cells and selective recruitment of ICAM-1 negative osteoblasts to counterbalance osteoclastic activity.

Trabecular thickening in subchondral bone is not always accompanied by increased bone mineralization but by osteoid volume increases^{38,39}. Osteoblasts express multiple adhesion molecules among which ICAM-1 and vascular cell adhesion molecule-1 play a pivotal role in osteoclastogenesis by producing bone resorbing cytokines through cell adhesion⁴⁰. Further, the membrane bound osteoclast differentiation factor provides a signal essential to osteoclast progenitors for their differentiation into osteoclasts³. These studies indicate that there appear to be at least 2 subpopula-

tions of osteoblasts: one induces osteoclastogenesis or bone resorption, while the other is involved in bone matrix secretion or bone formation; moreover cell adhesion of osteoblasts biases their functions toward inducing osteoclastogenesis rather than bone formation. This is an indication of abnormal mineralization^{41,42} as illustrated by the appearance of bone cysts in late OA, therefore suggesting that a dysregulation of bone remodeling may also be part of the disease. This would support the concept of a bone cell defect in high metabolic OA and, in some cases of fTHR from OA patients, may indeed be a selective ICAM-1+ osteoblast recruitment as suggested by Tanaka, *et al*⁵.

Previous *in vitro* studies of macrophages challenged with particles might have underestimated the dynamic interaction of macrophages and osteoblasts in response to inflammatory wear materials. Our study provides key evidence of altered metabolism in osteoblasts of fTHR-O and high metabolic OA. This, in turn, suggests that a subgroup of patients are prone to massive osteolysis and that we need to further broaden our knowledge of these patients in order to prevent early failure of prostheses. Elucidating the differences between normal, OA, and fTHR osteoblasts may also lead to identifying an alternative or complementary pathway in the pathophysiological mechanism(s) involved in OA and osteolysis in THR loosening.

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