The Characterization of Cytokines in the Interface Tissue Obtained From Failed Cementless Total Hip Arthroplasty With and Without Femoral Osteolysis

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The histologic, biochemical, and immunohisto-logic characteristics of the interface membranes surrounding the femoral component of failed cementless total hip arthroplasty (THA) in patients with (Group I) and without (Group II) radiographic evidence of focal endosteal erosion (osteolysis) were studied. Group I membranes had more macrophages and small particles of polyethylene debris in the membrane, but both groups had similar amounts of metal particles. A greater activity level of interleukin-1 (IL-1), tumor necrosis factor (TNF), and interleukin-6 (IL-6) was seen in the culture supernatant of the membranes from Group I than in that of Group II. Group I membranes also had more cells (macrophages, fibroblasts, and endothelial cells) that stained positively with anti-IL-6 antibody. These results suggest that IL-6, IL-1, and TNF play a role in the focal femoral osteolysis observed in patients with failed cementless hip prostheses.

Cementless total hip arthroplasty (THA) has become an accepted alternative to cemented THA in patients who are relatively young or active. This procedure is based on the concept of biologic fixation through bone ingrowth into the porous implant surface. In many cases, however, such implants have loosened.4,18,22-24 Radiographic analysis of the interface between the failing implant and bone generally shows a widening radiolucent zone with a variable amount of bone resorption on the endosteal surface of the femur.9,18,22 This process of femoral osteolysis has been observed and studied in cemented and cementless femoral components.4,7,18,22,23 Goldring et al.7 demonstrated that the supernatant fluid from the interface tissue obtained from patients with failed cemented implants produced significant levels of collagenase and prostaglandin E2 (PGE2). They proposed that these chemical mediators are associated with the process of failure in cemented implants when compared with pseu-docapsular tissue obtained from patients during revision surgery. Kim et al.14 recently found significantly higher levels of collagenase, PGE2, and IL-1 in the culture media of membrane tissue obtained from patients with failed cementless and cemented femoral components.14 Findings by these and other investigators suggest that these biochemical factors contribute to the process of bone osteolysis and aseptic loosening seen in either failed cemented or cementless THA.4,7

It is well known that osteoclastic bone resorption is stimulated by several biochemical
factors, including collagenase, PGE$_2$, IL-1, tumor necrosis factor alpha (TNF-$\alpha$), and IL-6. Interleukin-6 is a major cytokine involved in the inflammatory response and is secreted by several cell types, including T lymphocytes, B lymphocytes, macrophages, fibroblasts, and endothelial cells.

This study was undertaken to better understand the importance and presence of these important mediators of bone resorption in interface membranes that were obtained from patients with failed cementless THA that exhibited radiographic evidence of focal femoral endosteal erosion or osteolysis (Table 1).

**MATERIALS AND METHODS**

Between June 1990 and April 1992, 87 interface membranes were obtained from 87 patients undergoing revision surgery for failed cementless THA. Among these, 28 membranes were harvested from 28 patients who had undergone surgery for a failed cementless femoral component made of a titanium alloy. The indications for revision surgery included thigh pain and radiographic findings of implant subsidence. In addition to showing implant loosening in all patients, review of the radiographs showed discrete areas of femoral osteolysis in 14 patients (Fig. 1). Patients were classified based on the presence (Group I, n = 14) or absence (Group II, n = 14) of this osteolysis to clarify the histologic characteristics of failed cementless hip prostheses with focal femoral osteolysis and the contribution of various biochemical factors to this process. Although the socket liners were exchanged in many cases, acetabular osteolysis was not seen. So, visible polyethylene wear was noted in 20 cases: 12 patients in Group I, and eight patients in Group II. Serial radiographs were compared according to the femoral zones described by Gruen et al. The femoral endosteolytic lesions were evaluated according to Huddleston’s criteria. The anteroposterior radiographs showed the following distribution of focal osteolysis in Group I: multifocal in five patients (Grade IV, five patients), proximal (Gruen Zones 1, 7) in seven (Grade III, three patients; Grade IV, four patients), and distal (Gruen Zones 3, 5) in two (Grade I, one patient; Grade II, one patient). Group I had a younger mean age at the time of revision, but there were no significant differences between groups in the gender ratios, diagnoses, or intervals between the first and second operations. All implants were made of a titanium alloy, and all of the femoral heads were made of cobalt alloy. Implant stability was examined manually during the revision operation, and all exhibited visible motion at the bone–implant interface under manual pressure. In two cases, porous pad delamination had occurred. In all of the 28 patients, the preoperative aspirates and intraoperative cultures were negative. All patients had stopped taking nonsteroidal antiinflammatory agents at least two weeks before surgery.

The interface membrane from each of these patients was harvested and prepared for biochemical, histologic, and immunohistochemical analysis. The specimens were taken from the patients in Group I after visual and radiographic localization of the focal area of osteolysis at the time of revision surgery. Tissue was obtained from the outer surface of the pseudocapsules of ten additional patients undergoing revision THA to serve as control specimens and were prepared for identical biochemical and histologic analysis.

The tissue culture was prepared by the method described by Goldring et al., with some modifications for biochemical analysis. Specimens were cut

<table>
<thead>
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<th>TABLE 1. Patient Profiles</th>
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<tr>
<td><strong>Focal Osteolysis</strong></td>
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<td>Positive</td>
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<td>n</td>
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<td>Male:female</td>
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<td>Mean age at revision (years)</td>
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<td>Patients &lt;60 years old at revision</td>
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<tr>
<td>Diagnosis</td>
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<tr>
<td>Osteoarthrosis</td>
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<tr>
<td>Avascular necrosis</td>
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<tr>
<td>Congenital dislocation of the hip</td>
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<td>Old slipped femoral epiphysis</td>
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<td>Fracture</td>
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<td>Perthes’ disease</td>
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<td>Time to failure (months)</td>
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<tr>
<td>Type of implant</td>
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<td>HGP (Zimmer)</td>
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<td>Omnifit (Osteonics)</td>
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<td>Findings in implants at revision</td>
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<tr>
<td>Wear of UHMWPE</td>
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<td>Pad delaminations</td>
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UHMWPE, ultra-high molecular weight polyethylene.
into approximately 5-mm³ fragments and were placed in a culture dish. Three dishes were prepared per patient studied. After wet weights were determined, 2 ml serumless Neuman-Tytell medium (GIBCO, Grand Island, NY) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (GIBCO) were added. Cultures were incubated at 37°C in an atmosphere of 5% carbon dioxide and 95% air for three days. Then, the culture media were recovered and stored at −70°C for measurement of collagenase, PGE₂, IL-1, TNF-α, and IL-6.

Bioactivity of collagenase was measured with the method described by Watanabe et al., using Type I collagen labeled with [³H]acetic anhydride (New England Nuclear, Boston, Massachusetts) as the substrate. One unit collagenase activity degrades 1 μg per minute of substrate at 35°C. These results were expressed as units of proteinase per gram of tissue. The amount of PGE₂ was measured by radioimmunoassay using a commercially available kit (New England Nuclear, Boston, Massachusetts) according to the manufacturer’s instructions. Results were expressed in nanograms per gram tissue. To measure the bioactivity of IL-1, a bioassay using the method of Simon et al., was performed. Briefly, the test solutions were added to the EL-4 murine T-cell line in the presence of the ionophore A 23187. The medium conditioned by the EL-4 cells then was added to cultures of cytotoxic T lymphocyte line (CTLL)-20 cells, which require IL-2 for growth. These cells demonstrated a proliferative response, which was measured by the incorporation of [³H]-thymidine into TCA-insoluble material. Results were expressed in units per gram of tissue weight. The TNF-α and IL-6 were measured by an enzyme-linked immunosorbent assay with a commercially prepared kit according to the manufacturer’s instructions. Briefly, 200 μl of the culture media were added to 96-well microplates coated with mouse anti-human IL-6 or TNF-α monoclonal antibodies (first antibody). After two hours’ incubation at room temperature (IL-6), or at 37°C (TNF-α), the wells were washed, and 200 μl horseradish peroxidase-conjugated goat anti-human IL-6 or TNF-α polyclonal antibodies were added. After an additional two hours’ incubation at room temperature (IL-6), or at 37°C (TNF-α), the wells were washed again and incubated at room temperature for 20 minutes with chromogen (a mixture of H₂O₂ and tetramethylbenzidine). Then, 50 μl 2N H₂SO₄ was added to stop the reaction, and the optical density at 450 nm was measured using a microtiter reader. Results were expressed in nanograms per gram of tissue weight. Each assay was performed twice.

For histologic analysis, the interface membranes were fixed with 10% buffered formalin and embedded in paraffin. Sections were cut (5 μm thick) and stained with hematoxylin and eosin for histologic examination. A qualitative and semi-quantitative analysis of tissue characteristics was performed by using Mirra’s grading system as modified by Kim et al. For the immunohistologic analysis, the interface membranes were placed in OCT compound (Lab-Tek Products, Naperville, IL) and stored at −70°C until cut. Sections were cut (7 μm thick) on a cryostat, then dried overnight and fixed in cold
acetone (4°) for ten minutes. The fixed sections were incubated with 0.3% hydrogen peroxide for ten minutes to block endogenous peroxidase activity and then treated with normal horse or goat serum for 30 minutes before incubation with the first antibodies. Mouse monoclonal antibodies against B lymphocytes, T lymphocytes, macrophages (DAKOPATTS, Glostrup, Denmark), and rabbit polyclonal antibodies to IL-6 (CALBIOCHEM, La Jolla, CA) were diluted appropriately in phosphate-buffered saline (PBS; 10 mmol/l phosphate buffer, pH 7.4, 140 mmol/l NaCl) and used as the first antibodies. After incubation with the first antibodies for 45 minutes, the section was exposed to biotinylated anti-mouse or anti-rabbit immunoglobulin for 45 minutes and then to avidin-biotin-peroxidase complex (Vectastain, Burlingame, California) for 30 minutes. Finally, the site of peroxidase binding was disclosed with 3,3'-diaminobenzidine tetrahydrochloride (Accurate, Westbury, New York). The sections then were counterstained with hematoxylin. Between each step, the slides were washed three times for five minutes with PBS. Controls were performed as described, except for the omission of the first antibodies.

Statistical analysis was performed using Student's t-test, and p < 0.05 was considered to be statistically significant.

RESULTS

HEMATOXYLIN AND EOSIN STAINING OF THE MEMBRANE

The microscopic findings in the two groups were similar. A thin layer consisting of synovial-like cells or fibrin strands was observed adjacent to the implant surfaces. Beneath this layer lay an organized, dense connective tissue layer with sheets of macrophages, scattered foreign-body giant cells, and large accumulations of wear debris (including metal and polyethylene debris) were seen. Small polyethylene particles, sheets of macrophages that had phagocytized small polyethylene particles, and a proliferation of capillaries were seen adjacent to the bone side more commonly in Group I than in Group II. In contrast, sheets of fibroblasts with less frequent macrophages were more prevalent in Group II. Lymphocytic aggregates were rarely observed in either group.

ANALYSIS OF CHEMICAL MEDIATORS

As shown in Figure 2, membranes obtained from both groups released significantly high levels of IL-6, collagenase, PGE₂, IL-1, and TNF-α into the culture media than did the control pseudocapsular tissues. Furthermore, IL-1, IL-6, and TNF-α activity levels in Group I were significantly higher than in Group II, although the activity of IL-1 and TNF-α was much lower than that of IL-6. Collagenase activity and PGE₂ concentrations were not significantly different between the two groups, however.

IMMUNOHISTOCHEMICAL ANALYSIS

Analysis of the membranes showed that an infiltrate of anti-IL-6 positive cells were seen in Group I more frequently than in Group II (Fig. 3). Numerous macrophages, relatively large numbers of fibroblasts, some endothelial cells, but few T lymphocytes, and few B lymphocytes were stained with anti-IL-6 antibody in Group I (Fig. 4); the T and B lymphocytes were identified by immunostaining of serial sections using anti-CD 3 and anti-CD 22 monoclonal antibodies, respectively. In contrast, anti-IL-6 positive cells in Group II were limited to fibroblasts, small numbers of macrophages, few endothelial cells, few T lymphocytes, and few B lymphocytes. Group I generally had more cells (macrophages, fibroblasts, and endothelial cells) stained positively with anti-IL-6 antibody than did Group II.

DISCUSSION

Historically, the failure of THA has been considered primarily the result of biomechanical problems. It has become increasingly apparent, however, that interface membranes obtained from failed implants can produce biochemical substances that contribute to the bone resorption and the osteolysis associated with aseptic loosening of total hip components. This process can occur in the aseptic loosening of cemented implants
Clinical Orthopaedics and Related Research

**A** Collagenase (unit/g)

- Group 1 (n=14): 6.1 ± 3.0
- Group 2 (n=14): 6.0 ± 3.4

**B** IL-1β (unit/g)

- Group 1 (n=14): 10.5 ± 3.0
- Group 2 (n=14): 4.1 ± 5.3

**C** TNF-α (ng/g)

- Group 1 (n=14): 3.9 ± 2.0
- Group 2 (n=14): 2.1 ± 1.8

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**Prostaglandin E2 (ng/g)**

- Group 1 (n=14): 6097.1 ± 3518
- Group 2 (n=14): 6000 ± 2640

**IL-6 (ng/g)**

- Group 1 (n=14): 274.6 ± 120.5
- Group 2 (n=14): 134.3 ± 72.5
or of uncemented implants that are stable and ingrown or unstable and loose.\textsuperscript{18}

Examination of the histologic studies showed large amounts of metal and polyethylene debris and a nonspecific chronic inflammatory reaction in tissue taken from between the failed titanium implant and bone.\textsuperscript{1} The continual release of such debris from the joint and bone-implant interface can result from abrasion of the articulation between the femoral head and the polyethylene acetabulum as well as the micromotion of the prosthetic stem in the medullary canal. The debris then can be distributed throughout the joint and interface tissue.\textsuperscript{16}

The histologic findings in this study were similar in the membranes from patients with and without focal osteolysis. Those with focal osteolysis had a greater number of macrophages that phagocytized small polyethylene debris, whereas patients without focal osteolysis had more fibroblasts. These findings are consistent with those of Santavirta \textit{et al.},\textsuperscript{23,24} who recently observed an immunopathologic response in aggressive granulomatoses in cemented and cementless arthroplasties that included many macrophages but few fibroblasts.

Recently, Maloney \textit{et al.}\textsuperscript{19} reported that 90\% of polyethylene particles that were obtained from human interface membranes were smaller than 1 \textmu m, approximately 0.6 to 0.7 \textmu m. This report has indicated that many of these polyethylene particles were too small to be resolved with light microscopy; therefore, microscopic analysis may not provide an accurate estimate of the amount of wear debris in the membranes.

Because there were no significant differences between the two groups of patients in the quantities or the size of metal debris nor in the type of implant biomaterials, these histologic differences may represent a differing host reaction to metal particulates or the quantities of small polyethylene debris not visible with light microscopy. It is also possible that younger patients may produce a stronger foreign-body reaction.\textsuperscript{2} Furthermore, because there was such disparity between both the average age and the quantities of small polyethylene debris in Group I and Group II, one could argue that younger patients are more active, thus causing more micromotion at the implant site and increased
FIGS. 4A–4C. Immunohistochemical staining (anti–IL-6 antibody) of the membranes from a patient with focal osteolysis demonstrated anti–IL-6-positive macrophages (A), fibroblasts (B), and endothelial cells (C).

Polyethylene debris, which, in turn, may promote focal osteolysis and loosening.

Both titanium alloy and cobalt chrome implants, whether loose or well fixed, demonstrated increased capsular and synovial fluid ion levels. The release of metal particles and metal ions may be related to the large surface area of porous-coated implants that is in direct contact with tissue. There could have been significant differences, undetectable by this analysis, between wear- and corrosion-induced soluble metal ions in the two groups of patients.

It is well known that several biochemical mediators, including various cytokines, contribute to inflammatory reactions. Biochemical analyses showed that IL-1, TNF-α, and IL-6 activities were significantly higher in patients with focal osteolysis than in those without this feature, but there were no significant differences in the amounts of collagenase and PGE₂. Because more macrophages were observed in patients with focal osteolysis and more fibroblasts in those without, the difference between macrophages and fibroblasts in the secretory activity of collagenase and PGE₂ might result in an insignificant difference between the collagenase and PGE₂ levels in the two groups of patients. Evans et al. showed that synovial fibroblasts acti-
vated by wear particles secreted more than five times as much collagenase as the same number of the macrophages in vitro. Interleukin-1 and TNF-α induce not only the production of IL-6 by macrophages, fibroblasts, and endothelial cells, but also the proliferation of fibroblasts and endothelial cells. Furthermore, IL-1 and TNF-α are stimulators of osteoclastic bone resorption. It recently was documented that IL-6 induces osteoclastic bone resorption directly. Biochemical and immunohistochemical results suggest that macrophages, fibroblasts, and endothelial cells in the membranes react with metal or polyethylene debris and produce IL-6, which directly stimulates osteoclasts in patients with focal osteolysis. Because IL-1 and TNF-α both stimulate osteoclastic bone resorption and induce IL-6, another possibility is that IL-1 and TNF-α may contribute to the osteoclastic bone resorption directly as well as indirectly through the induction of IL-6 production by macrophages, fibroblasts, and endothelial cells. It is unlikely that B lymphocytes and T lymphocytes contribute to IL-6 production, because these two cell types were rarely observed in the current study. These observations suggest that significant levels of IL-6 activity were produced through a nonspecific chronic inflammatory response to small polyethylene debris rather than through the lymphocyte-mediated immune response. Luckey and Venugopalan reported that titanium, aluminum, and vanadium were recognized neither as whole antigens nor as haptens. Moreover, small polyethylene debris that were phagocytized by macrophages might explain why more macrophages were found in membranes obtained from patients with focal osteolysis, given that there were greater quantities of small polyethylene debris in this group.

Based on the findings of this study, the authors believe that cytokines including IL-6, IL-1, and TNF-α that are produced by macrophages, fibroblasts, and endothelial cells in a nonspecific chronic inflammatory response to small polyethylene debris may contribute to the focal osteolysis seen in patients with an uncemented titanium alloy femoral arthroplasty.

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