Effect of PMMA particles and movement on an implant interface in a canine model

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The pathogenesis of aseptic loosening of total joint prostheses is not clearly understood. Two features are associated with loosened prostheses, namely, particulate debris and movement of the implant. While numerous studies have evaluated the cellular response to particulate biomaterials, few have investigated the influence of movement of the implant on the biological response to particles. Our aim was therefore to test the hypothesis that excessive mechanical stimulation of the periprosthetic tissues induces an inflammatory response and that the addition of particulate biomaterials intensifies this.

We allocated 66 adult Beagle dogs to four groups as follows: stable implants with (I) and without (II) particulate polymethylmethacrylate (PMMA) and moving implants with (III) and without (IV) particulate PMMA. They were then evaluated at 2, 4, 6, 12 and 24 weeks.

The stable implants were well tolerated and a thin, fibrous membrane of connective tissue was observed. There was evidence of positive staining in some cells for interleukin-6 (IL-6). Addition of particulate PMMA around the stable implants resulted in an increase in the fibroblastic response and positive staining for IL-6 and tumour necrosis factor-alpha (TNF-α). By contrast, movement of the implant resulted in an immediate inflammatory response characterised by large numbers of histiocytes and cytokine staining for IL-1β, TNF-α and IL-6. Introduction of particulate PMMA aggravated this response. Animals with particulate PMMA and movement of the implant have an intense inflammatory response associated with accelerated bone loss.

Our results indicate that the initiation of the inflammatory response to biomaterial particles was much slower than that to gross mechanical instability. Furthermore, when there was both particulate debris and movement, there was an amplification of the adverse tissue response as evidenced by the presence of osteolysis and increases in the presence of inflammatory cells and their associated cytokines.

Since the first reports of the failure of total joint prostheses, there has been considerable debate as to the role of biological and mechanical factors in the progression of aseptic loosening of the components. Based on an accumulation of recent evidence, there is a growing consensus that it is the biological response to particulate biomaterials which initiates the loosening process. Large quantities of polyethylene (PE), polymethylmethacrylate (PMMA) and metal alloy particles have been identified in periprosthetic tissues. Analysis has indicated that there is a preponderance of submicron particles which can be easily phagocytosed by the cells located within the membrane. Particles of implant biomaterials have been shown to have the capacity to stimulate the release of inflammatory mediators from the cells found within the periprosthetic membranes of failed total joint arthroplasties.

Mechanical factors undoubtedly contribute to the loosening process. As clinical experience has shown, failure of the mechanical integrity of the interface and the subsequent gross movement of the component results in catastrophic bone loss. Improvements in surgical technique, including improved instrumentation for implantation and cementing techniques, have led to a dramatic reduction in mechanical failures. Harris and his colleagues have proposed that mechanical factors may contribute to the loosening process. They suggest that debonding of the cement mantle may result in the creation of an ‘effective joint space’ between the mantle and the implant which can then act as a conduit for wear particles and inflammatory mediators. Additionally, during the development of mechanical failure (debonding, cracking), it is likely
that the stresses experienced by the bone at the interface are altered which may also have biological consequences.

There is little information concerning the events which occur between the initial biological response after implantation of the prosthesis and the end-stage diagnosis of aseptic loosening. By the time that most patients present with a failed implant they are in the ‘late stages’ of this adverse response. Earlier detection of aseptic loosening is limited by the lack of symptoms in the initial stages. Small radiological changes cannot be detected by current techniques. Furthermore, the biological response in clinically successful prostheses is incompletely characterised. Only a few studies have reported the analysis of cadaver specimens. Since human material is not readily available at all stages of the wearing of an implant, animal models are necessary to study the cellular and biochemical responses to wear particles which are created even in successful prostheses. We have therefore developed and characterised an animal model of an aseptically loosened cemented implant. Several features of this animal model make it a useful tool: 1) it allows observation of defined time points including immediately after loosening has occurred; 2) histological examination includes both the soft-tissue membrane as well as the underlying bone structure; and 3) the effects of particulate biomaterials and movement can be examined separately and in combination. Our aim was to evaluate the cellular and cytokine response of the animal model to particulate biomaterials and movement of the implant over a period of time ranging from two weeks to six months.

Materials and Methods

We used skeletally mature, heartworm-free, purpose-bred Beagles. There were 58 females and eight males and their weight ranged from 6.8 to 17.2 kg. Skeletal maturity and the absence of previous or current pathology were confirmed radiologically. The animals were housed according to the applicable laws and regulations relating to laboratory animals.

Experimental design. This animal model is a modification of that first described in 1987 by Lennox et al. A PMMA-coated, polyethylene (PE) rod is placed in a tibial defect. The opposite end is fixed within the distal femur. Flexion and extension of the knee create a pistoning of the implant within the tibial defect (Fig. 1).

The 66 dogs were allocated to four groups as follows: group I, stable implant, no particles added; group II, stable implant, PMMA particles added; group III, moving implant, no particles added; and group IV, moving implant, PMMA particles added. They were evaluated at 2, 4, 6, 12 and 24 weeks. A minimum of three animals was included in each subgroup. Any animals with non-functional ‘moving’ implants, as determined radiologically, were replaced.

Description of the implant. Ultra-high-molecular-PE rods (0004-5-100) (Howmedica Inc, Rutherford, New Jersey), were machined into a barbell shape (Fig. 2). The thin portion of the barbell had an outer diameter of 2 mm to give the flexibility needed for flexion and extension of the stifle joint. The outer diameter of the barbell-shaped ends was 5 mm. To create a uniform layer of PMMA around the implant, one barbell-shaped end of the rod was inserted into a glass tube (inside diameter, 7 mm) containing polymerising bone cement (Low Viscosity Cement; Zimmer, Warsaw, Indiana). The PE rod was placed within the centre of the cement mantle. A metal pin was embedded into the PMMA to give a radiological marker. Once polymerised,
the implant was slid out of the tube. All implants were autoclaved before implantation.

**Operative technique.** The animals were prepared for surgery according to the usual standard protocol for aseptic surgical procedures. They were preanaesthetised with Pentothal (Abbott Laboratories, North Chicago, Illinois) (30 mg/kg) and anaesthesia was maintained with Ethrane (Anaquest, Madison, Wisconsin) introduced with O₂/N₂O. Intramuscular gentamicin (4 mg/kg) was given preoperatively and for two days after the operation.

A medial parapatellar approach was used and an incision made parallel to the medial border of the patella and patellar ligament. The patella was then luxated laterally. A drill hole was made in the intercondylar eminence just anterior to the horns of the menisci and the attachment of the anterior cruciate ligament. A pilot drill hole (4 mm) was made over a 0.045 Kirschner wire inserted into the narrow cavity of the proximal tibia. Using sequentially larger drill bits, the defect was enlarged in the desired alignment to a final diameter of 4.7 mm. If the animal was assigned to one of the particle groups, 0.1 g of particulate PMMA was placed in the tibial defect. Particulate PMMA was generated by milling polymerised bone cement (Zimmer, Warsaw, Indiana). The size of the particles ranged from submicron to 500 μm and 12% were below 12 μm, which is the upper limit of phagocytosis as determined in vitro for macrophages. For the distal femoral fixation, a 4.7 mm drill hole was made in the femur within the trochlear sulcus just anterior of the insertion of the posterior cruciate ligament. The implant was placed into both the tibial and femoral defects. The anterior end of the PE rod was fixed within the femoral defect using a 0.054 Kirschner wire placed transcortically. Flexion and extension of the knee caused a pistoning of the implant within the tibial defect (Fig. 1). For the groups with stable implants, these were sectioned just above the PMMA coating of the PE barbell. Non-moving implants were positioned within the tibial defect below the articular surface. With the patella relocated, the wound was closed in layers. A loose bandage was applied to the affected joint. Mediolateral radiographs were taken with the knee in full extension and also flexed maximally. The mean maximal excursion of the implant as measured on the radiograph was 3.3 mm, but was mainly below 2.7 mm.

The animals received Talwin (Sanofi Winthrop Pharmaceuticals, New York) (3 mg/kg) as postoperative analgesia as needed. They were allowed to bear weight on the operated joint as tolerated. Wound healing and weight-bearing status were assessed and recorded daily.

**Processing of the specimens.** At the predetermined time period, each animal was euthanised by an overdose of phenobarbital. Radiographs were taken as previously described. A swab culture was taken of the joint and analysed to rule out infection, in accordance with the guidelines of McDonald, Fitzgerald and Chao. With the moving implants, we made a final assessment as to whether the implant remained intact. The intact thin portion of the PE rod was then sectioned. The proximal tibia was cut below the tibial tuberosity. In order to hemi-sect the proximal tibia, saw cuts were made through the cortical wall and the cut was continued to the level of the defect with an osteotome. The implant could then be removed from its tissue bed with the least amount of disruption of the interface. Each hemi-sected specimen was photographed before and after removal of the implant. If present, a sample of the soft-tissue interface membrane was removed and snap-frozen in optimum cutting temperature (OCT) compound (Tissue-Tek; Sakura Finetek USA Inc, Torrance, California) with liquid nitrogen to be analysed by immunohistochemistry. One half of each bone specimen was placed into either 70% ethyl alcohol or 10% buffered formalin for fixation.

**Immunohistochemistry.** Serial sections (5 μm) were made from each frozen membrane specimen using a cryostat (Leica, Dearfield, Illinois). They were mounted on poly-L-lysine slides and fixed with alumina-filtered acetone (10 minutes). The sections were washed twice with phosphate-buffered saline (isotonic PBS; pH 7.4) for five minutes each and blocked with 0.3% H₂O₂ for 30 minutes at 37°C. They were again washed three times for five minutes each with PBS. Incubations with all subsequent reagents were carried out in a humidified, darkened, plastic chamber. Each specimen was incubated with the primary antibody at 4°C overnight. The optimal concentration of the antibodies was predetermined using serial dilutions. The primary antibodies used included interleukin-1 beta (IL-1β, 1:100, polyclonal, rabbit anti-human; Endogen, Woburn, Massachusetts), tumour necrosis factor-alpha (TNF-α, 1:100, polyclonal, rabbit anti-human; Endogen) and IL-6 (1:100, monoclonal, rabbit anti-human; Upstate Biotechnology Inc, Lake Placid, New York). After incubation with the primary antibody, the slides were washed with PBS (three times, five minutes/wash), and the biotinylated secondary antibody (goat anti-rabbit, Vectastain KIt; Vector Laboratories Inc, Burlingame, California) was applied for one hour at 37°C. This was followed by three washes with PBS and then incubation with avidin-biotin complex (VectaStain Kit; Vector Laboratories Inc) for one hour at 37°C. After washing the slides again, 3,3-diaminobenzidine tetrahydrochloride was applied to each section for five minutes at room temperature. The slides were washed with water (five minutes) and then 0.5% copper sulphate solution was applied (two minutes). All slides were counterstained with Mayer’s haematoxylin. Additional slides were included as ‘controls’ for technique (omission of primary or secondary antibodies) and tissue (staining of spleen samples) specificity. A routine slide stained with haematoxylin and eosin was obtained from each frozen specimen.

All slides were coded so that they could be read without knowledge of the group or time assignment. They were viewed under a bright field using a Leitz Laborlux S microscope (Leitz, Heerbrugg, Switzerland). Each slide...
Representative radiographs for each of the groups. Figures 3a and 3b – Group I (no particles, stable implant), a) at two weeks and b) at six months. Figures 3c and 3d – Group II (particles, stable implant), c) at two weeks and d) at six months. Figures 3e and 3f – Group III (no particles, moving implant), e) at two weeks and f) at six months. Figures 3g and 3h – Group IV (particles, moving implants), g) at two weeks and h) at six months. Arrows identify the edge of the defect.
Photomicrographs for each of the groups. Figures 4a and 4b – Group I (no particles, stable implant), a) at two weeks and b) at six months. Figures 4c and 4d – Group II (particles, stable implant), c) at two weeks and d) at six months. Figures 4e and 4f – Group III (no particles, moving implant), e) at two weeks and f) at six months. Figures 4g and 4h – Group IV (particles moving implants), g) at two weeks and h) at six months (*, location of implant, removed to process; modified Stevenol’s blue stain ×250).
was graded as follows: 0, no stain; 1, focal staining, limited distribution; 2, numerous focal areas of staining; 3, disseminated staining, but some regions unstained; 4, staining throughout the section. Photographs were taken of each slide using a Leitz automated camera system. 

Undecalcified sections. The fixed-tissue specimens were embedded in methylmethacrylate according to the method of Emmanuel et al. Thin sections were cut and ground using the Exact System saw and grinder (EXACT Technologies, Oklahoma City, Oklahoma). Each slide was then surfaced stained with modified Stevenol’s blue stain (no etching) and coded to allow histological evaluation in a blinded fashion.

Results

In all of the dogs healing was uneventful. After three days most animals could bear weight partially and had returned to full weight-bearing by seven days. Five of the moving implants failed during the course of the study and were excluded from the analysis. They broke at the thin portion of the rod. As stated previously, these animals were replaced in the study. There was no evidence of deep infection in any of the animals.

Group I (no particles, stable). There was no detectable radiological evidence of osteolysis surrounding the stable implants for any of the time periods evaluated. Regeneration of bone was observed across the defect up to the surface of the implant by two weeks and continued up to six months (Figs. 3a and 3b). Post-mortem removal of the implant revealed an exact impression of the implant. In the shorter time periods (2, 4 or 6 weeks) gross inspection did not show any evidence of a membrane. Histological examination, however, revealed an encapsulating membrane a few cell layers thick (Fig. 4a). The underlying bone appeared to be mature with an adequate blood supply. By six months, the membrane had thickened, was more fibrous in nature and was organised into sheaths parallel to the surface of the implant (Fig. 4b). As shown in Table I, the membrane had formed next to the host bone (Fig. 4c). By six months, it had increased dramatically in size, but remained primarily fibroblastic in composition (Fig. 4d). As with the previous group, there was minimal staining for IL-1ß but, unlike group I, there was occasional evidence of staining for TNF-α which was focally distributed. Membranes from this group were also positive for IL-6 which was distributed throughout the membrane (Fig. 5b). As with group I, there was no radiological or histological evidence of osteolysis. The bone contained a degree of cellularity similar to that of group I.

Group III (no particles, moving implant). The radiographs confirmed that the implants were intact just before post-mortem (Figures 3e and 3f). In contrast to the previous groups, there was evidence of a gap surrounding the implant at all the time periods studied. When comparing the immediate postoperative radiographs with those at post-mortem, the gap surrounding the implants appeared to have enlarged. In the six-month animals, there was radiological evidence of ‘cystic’ osteolysis. Gross observation showed a thick soft-tissue membrane which was apparent at all time periods. The underlying bone was completely covered with the membrane and the bone surface was not observable. As shown in Figures 4e and 4f, the histological appearance of the membrane was different from that from around the stable implants (with and without particulate PMMA). Although fibrous tissue remained present, there were increased numbers of osteoclasts and macrophages at the bone-membrane interface. Neovascularisation was evident. Osteoblastic activity and seams of osteoid were prevalent throughout the bony interface. Evidence of osteoclastic activity was apparent, but osteoclasts were not common. Both IL-1ß and TNF-α were localised within the membranes and appeared to be primarily associated with the macrophages. IL-6 was clearly present and was associated with both fibroblasts and macrophages (Fig. 5c).

Group IV (particles, moving implant). As shown in Figures 3g and 3h, the radiographs from the different time periods revealed an enlarging gap surrounding the implants. The radiolucencies were indistinguishable from those seen in human ‘cystic’ radiological osteolysis. At post-mortem, as with group III the membranes had developed by two weeks and had a pseudosynovial-like appearance. The bone

<table>
<thead>
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<th>Group</th>
<th>IL-1ß</th>
<th>TNF-α</th>
<th>IL-6</th>
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<tr>
<td>I (stable, no particulate PMMA)</td>
<td>1</td>
<td>0</td>
<td>1 to 2.5</td>
</tr>
<tr>
<td>II (stable, particulate PMMA)</td>
<td>1</td>
<td>0</td>
<td>1 to 2.5</td>
</tr>
<tr>
<td>III (moving, no particulate PMMA)</td>
<td>0 to 2.0</td>
<td>0 to 2.0</td>
<td>0.5 to 2.0</td>
</tr>
<tr>
<td>IV (moving, particulate PMMA)</td>
<td>0.5 to 1.0</td>
<td>0 to 1.5</td>
<td>1.0 to 3.0</td>
</tr>
<tr>
<td>Human</td>
<td>4</td>
<td>4</td>
<td>4</td>
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Table I. Semiquantitative assessment of the immunohistochemical staining for cytokines at six months. For grading see text.
cavity no longer reflected the shape of the implant. Histo-logically, although the interface membrane contained both histiocytic and fibroblastic zones (Figs 4g and 4h) the quantity of tissue containing histiocytes had increased. Multinucleated cells were a more common occurrence. The membranes were highly vascularised. The bone interface appeared to be somewhat disrupted. Although there remained evidence of osteoblastic activity, osteoclasts were easier to locate than in the previous groups. The membranes continued to stain positively for IL-1ß, TNF-α, and IL-6 (Table I), but the staining was more widely distributed than in the previous groups (Fig. 5d).

**Cellular and cytokine profiles.** The results of the qualitative assessment of the cellular profiles for the four experimental groups at six months are shown in Table II. The character of the membranes from the interfaces of the stable implants (with or without particulate PMMA) was primarily fibroblastic. The membranes from the group-III animals contained both fibroblastic and histiocytic tissue. Although fibroblasts and macrophages (histiocytes) were evident in the membranes of the group-IV animals the interface tissues were primarily histiocytic.

<table>
<thead>
<tr>
<th>Group</th>
<th>Membrane*</th>
<th>Osteolysis</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>F+</td>
<td>-</td>
</tr>
<tr>
<td>(stable, no particulate PMMA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>F++</td>
<td>-</td>
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<tr>
<td>(stable, particulate PMMA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>F, H++</td>
<td>+</td>
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<tr>
<td>(moving, no particulate PMMA)</td>
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<tr>
<td>IV</td>
<td>H, F++++</td>
<td>+++</td>
</tr>
<tr>
<td>(moving, particulate PMMA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>H,F+++++</td>
<td>++++</td>
</tr>
</tbody>
</table>

* F, fibrous; H, histiocyte, +, minimal; ++++ maximal

The cytokine profiles for the six-month animals for each of the experimental groups are shown in Table I. IL-6 was evident in all of the experimental groups. The results for IL-1ß and TNF-α were more variable. Although IL-1ß was present in the membranes from all groups, the median ranking was 1 out of a possible 4 for all of the groups. TNF-α was not detected in the membranes from the group-I animals, but was seen in most of the animals in the other
experimental groups. The membranes from the group-IV animals contained the widest distribution of cytokine immunostaining.

Discussion

In patients with aseptic loosening, tissue response at the prosthesis-bone interface is characterised by the infiltration of cells associated with chronic inflammation and by the loss of bone, i.e., osteolysis. It is not clear as to what triggers and/or accelerates the inflammatory response and which cells and cell products are responsible for the bony destruction. There are two consistent features of loosened components: 1) particles of biomaterials which are contained within the membrane; and 2) movement at the interface. For this reason, we sought to study the potential role of each of these factors, separately and in combination, on the development of an interfacial membrane using our animal model.

Biomaterial particles. Our results support the findings of Howie et al that intact PMMA implants are well tolerated by the host as shown by the presence of a thin fibrous interfacial membrane and bony encapsulation. When particles of PMMA were introduced to the interface surrounding a stable implant in our model, there was an increase in the amount of fibrous tissue at the interface. The response appeared to be relatively benign with marginal staining for inflammatory cytokines. Goodman, Fornasier and Keil reported a more inflammatory response to particles of PMMA with large numbers of histiocytes contained within a soft-tissue stroma. Differences between our results and those of Goodman et al may reflect differences in the design of the study, the PMMA particulates used (size, ground versus beads), or the animals used (dogs versus rabbits). There were also significant differences between the models. Goodman et al inserted the particulate PMMA into a 6 mm defect, while in our model the particulate PMMA was placed around a defect-filling, intact PMMA-precoated implant. Despite these differences, the results of both studies indicate that particulate biomaterials may play a role in the recruitment or proliferation of cells found within periprosthetic membranes.

In regard to the bony interface, we did not detect any adverse response to the host bone when particles were introduced around a stable cement plug. New bone filled the gap created at surgery and there was no evidence of cystic osteolysis. By contrast, others have shown that particles of biomaterials introduced into animal models have an impact on bone. Using a ‘bone harvest chamber model’, Goodman et al found that the addition of particulate PMMA as well as other types of biomaterial particle (HDPE, CoCr alloy) resulted in a decrease in net bone formation as compared with a control group. Howie et al showed in rabbits that the repeated introduction of PE particles into the joint created a response around a PMMA plug implanted into the distal femur consistent with the diagnosis of osteolysis. Interestingly, the PMMA particles used in their study ranged from 20 to 200 µm, a size generally considered to be too large to be phagocytosed. Again, the differences between our results and those of the other studies may reflect differences in the models and experimental designs.

Movement of the implant. Most laboratory studies of the micromovement of the implant have used cementless fixation. Aspenberg and Herbertsson evaluated the effect of movement on a titanium implant interface using a model which involved rotating the test surface 20 half-turns (180°) twice daily. A fibrous tissue membrane developed at the interface which increased in size with time. Schwendeman et al and Dowd et al characterised a model in which instability was created using an articulating joint between the proximal and distal stem of a titanium-alloy femoral component. Movement of the uncemented metal implant resulted in a biological response described as a “lush periprosthetic membrane between the bone and metal” which was “more cellular with sheets of macrophages and giant cells, and more debris”. Soballe et al and Bechtold et al developed a model in which loading of a PE-tipped titanium alloy implant by the knee resulted in movement of the implant at a level approximating 500 µm. The movement is stopped by removing a spring-loading mechanism. The results of their studies provide evidence that a histiocytic, fibrous membrane will develop within the interface around unstable, uncemented implants.

Our results suggest that the initial stability of the implant is also relevant to cemented implants. An interface membrane containing large numbers of histocytes and fibroblasts had developed in all animals by the two-week period. This was associated with what appeared to be an increase in the overall activity of the bone cells present at the interface and the presence of cytokines known to be involved in the inflammatory response.

Biomaterial particles and implant motion. It is clear from our results and others that animal models which incorporate both biomaterial particles and implant instability result in a radiological and histological picture strikingly similar to that seen in the clinical situation with catastrophic failure of orthopaedic implants. Using the model of Aspenberg and Herbertsson, Goodman et al found that PE particles and intermittent micromovement gave a highly fibrous stroma containing foreign-body cells and birefringent particles. Spector et al in a canine model using a loose, cemented femoral hip component and bone-cement particles, noted an expansion of the radiolucent zone surrounding the implant with time, up to seven months after operation. In agreement with the results of Spector et al, we found that radiolucent zones are readily apparent around an unstable implant which contains particulate biomaterials in the interfacial tissues. The histological appearance of the tissue within the cavity surrounding these models is consistent with the diagnosis of chronic inflammation.
Inflammatory mediators. Several substances which may modulate the inflammatory response associated with aseptic loosening have been shown to be elaborated by interface membranes surrounding failed human implants. These have included cytokines, eicosanoids, metalloproteinases and other enzymes. Similar findings have been demonstrated in animal models. Cuckler et al found a time-dependent change in the leukocyte number and the level of protease in response to particulates of titanium, PMMA, and titanium/PMMA. At one hour after implantation, the control and titanium groups had a higher activity of protease than the PMMA and PMMA/titanium groups. By six hours, this relationship had reversed with large increases in the activity of protease in the groups containing PMMA. Dowd et al noted that, after 12 weeks of implantation, there was an increase in the collagenase, PGE₂ and IL-1 as compared with a control group when particles (cobalt-chromium alloy, titanium alloy, and PE) were introduced into a gap surrounding a metal implant. Our study showed that the cytokines IL-1β, IL-6 and TNF-α were present at six months after implantation, but in very low concentrations, in the soft tissues surrounding stable implants with and without the addition of particles of biomaterials. This did not appear to have an effect on the surrounding bony tissues. Longer time periods, however, are needed to determine the long-term consequences of this finding.

Within the membranes from around moving cement-coated implants, we noted that there were higher levels of IL-1β, IL-6 and TNF-α as compared with the non-moving implants. Bechtold et al showed that moving implants resulted in a large increase in IL-6, a marginal increase in TNF-α, and a large decrease in TGF-β in the synovial fluid of the joints with moving implants. Dowd et al found significantly increased gelatinase, collagenase, PGE₂ and IL-1 levels in membranes surrounding unstable implants as compared with stable controls. Based on immunocytochemical localisation, the levels of cytokine were further increased with the addition of particulate PMMA into the defect around moving implants in our study. Spector et al reported that in their model (loose cemented component, PMMA particles) the interface was rich in macrophages with elevated levels of PGE₂ and IL-1-like activity. They also demonstrated that this membrane had the capacity to resorb bone using a calvarial model.

Our study has addressed the response to specific features surrounding the loosening process, namely, movement of the implant and particulate debris. As with the human studies, most animal studies have focused on the ‘end-stage’ of the loosening process. Although this profile may include the cytokines present throughout the pathogenetic process, it is also possible that the types and levels of cytokines change throughout the course of loosening. Overall, we noted that the level of cytokine release appeared to increase with longer durations of implantation for all of the cytokines studied. Each was barely detectable at the early time periods.

There are limitations to the animal model which we have used in our experiments. It is a post facto model in that it evaluates the response to a massive amount of particles, not an accumulation of particles or a progressive increase in movement. The model simulates an ‘all-or-nothing’ condition: the implants were either stable or subjected to a considerable degree of movement. It can, however, be used to determine the biological response to the implant once these situations have occurred singularly and in combination and to study the treatment of failed arthroplasties. One limitation of many of the other studies is that they tended to focus only on the characteristics of the soft-tissue membrane. A major advantage of animal models, however, as shown here, is the ability of the investigator to look at the relationship of this membrane to the activity of the underlying bone. This model has certain advantages over animal models using total joint prostheses. It is less expensive, has fewer uncontrollable variables, and yields reproducible results.

The results of all of the animal models for unstable implants show that the stability of the implant considerably influences the biological response to implants in bone. Therefore, like their cementless counterparts, the long-term success of cemented implants is also dependent on the initial stability of the prosthesis. At low levels of micromovement, it may also be possible that the mechanical environment influences the biological response. As a result of strains experienced at the interface, certain types of cell, particularly fibroblasts, may be recruited to the interface. This has been shown to be true for cementless prostheses; it may be true also for cemented prostheses. Several investigators have suggested that a fibrous membrane may act as a ‘bursa’ or ‘buffer’ to modulate the amount of strain experienced by the interfacial bone. If the development of the fibrous membrane is allowed to expand, it is likely to affect the mechanical integrity of the interface.

Do our results indicate that osteolysis can only occur with movement? Although this may be one interpretation of our data, it is probably an oversimplification. We propose that the biological response to biomaterial particles is much slower than that to gross mechanical instability. Our results are consistent with the hypothesis that once both particulate biomaterials and movement have occurred the inflammatory response is dramatically increased. It may be that the cells at the interface are ‘primed’ to respond to the effect of particulate biomaterials under certain mechanical environments. In other words, in response to increases in the stress and strain experienced at the implant interface, the cellular activity may be increased as indicated by increases in the levels of DNA and RNA and/or increases in the synthesis of certain proteins. Wang and Goldring suggested a “double-hit” hypothesis for the pathogenesis of aseptic loosening. Although their double hit involved two different particulate biomaterials, the concept can be expanded to include two different stimuli, one biological (particles) and the other mechanical (movement). In fact, defining...
the role of particulates and implant stability in the loosening process will have significant implications in determining the approaches used to reduce or to prevent osteolysis.

The authors acknowledge financial support from the NIEHS Training Grant ES 07141, the Good Samaritan Hospital Endowment, and the JHU Orthopaedic/Rheumatology Gift Fund. The authors thank Diane Banes Boulay, Chris Ellis, Corrine Young, Hilary Reidy, and Denise Opiškinski for their technical support. The authors would also like to thank Clara Ma, Debi Lewis and Debi Ross for their secretarial support.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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