Positive cytokine production in failed metal-on-metal total hip replacements

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Submitted 01-07-29. Accepted 02-03-07

ABSTRACT – Tissues surrounding failed conventional total hips have been shown to produce inflammatory cytokines that can induce osteoclastic bone resorption. We evaluated the cytokine profiles of tissues from 5 failed metal-on-metal total hip replacements. Serial frozen sections were stained using immunohistochemical and in situ hybridization techniques. Inflammatory and osteoclast-stimulating cytokines were noted in the tissues.

As compared to a group of 5 metal-polyethylene hip tissues, we found fewer CD68 positive macrophages, and lower levels of TGF-β and TNF-α, but no differences in CD3 positive lymphocytes, IL-1β, IL-6 and PDGF-α in the metal-on-metal tissues. This may be due, in part, to the presence of wear particles from sources other than the bearing surfaces. Thus, cytokines associated with bone resorption and implant loosening may occur in total hips despite the use of alternative bearing materials.

The pathogenesis of loosening of joint replacements continues to be a major focus of research in orthopaedics and many studies have found that polyethylene wear particles largely contribute to the process. Tissues surrounding failed total hips have been shown to produce inflammatory cytokines such as interleukin 1 and interleukin 6 that can induce osteoclastic bone resorption (Goldring et al. 1983, Horowitz et al. 1993, Jiranek et al. 1993, Goodman et al. 1998).

As a result, implant manufacturers have turned to alternative bearing materials for total hip replacement. Metal-on-metal (M-M) total hips were reintroduced in Europe a decade ago and are beginning to be used clinically in the USA. Hip simulator studies of modern metal-on-metal bearings have shown that they produce low volumetric wear (Chan et al. 1996, McKellop et al. 1996, Anissian et al. 1999), and this has been verified by wear measurements of retrieved components (Campbell et al. 1999, Sieber et al. 1999). However, there is little information from retrieval studies on the histological reaction to wear products formed in vivo. In particular, the ability of tissues to produce inflammatory cytokines is not known.

We assessed the histopathology of revised metal-on-metal total hips and compared the results with those from revised metal-on-polyethylene (M-PE) hip replacements, using immunohistochemical techniques for cytokines that have been reported to be associated with osteolysis around total hip replacements.

Material and methods

Periprosthetic tissue was collected from the acetabulum and the posterior superior capsule region of 5 hips at revision surgery. The patients had received M-M components (McMinn surface replacements, Corin, UK). These components were manufactured from a cobalt chromium molybdenum alloy and were affixed with cement on the femoral side, and without cement on the acetabular side (Table 1). The cause of failure (component loosening, neck fracture or mesh debonding) was associated with the production of bone cement, bone and/or metal particles. Light burnishing on the femoral surfaces reflected the presence of third body wear particles in each case.

Table 1. Clinical data

Case	Gender	Age	Time to revision (months)	Reason for revision	Type
1	F	56	15	Loosening	M-M
2	M	63	9	Fracture	M-M
3	M	42	26	Mesh debonding	M-M
4	M	50	7	Loosening	M-M
5	F	28	25	Loosening	M-M
6	M	36	126	Osteolysis	M-PE
7	M	38	85	Osteolysis	M-PE
8	M	65	94	Synovitis	M-PE
9	M	38	103	Osteolysis	M-PE
10	F	56	100	Loosening	M-PE

McMinn (Corin, UK), PSR (Porous Surface Replacement, Zimmer, Warsaw IN, USA)

For comparison, tissues from 5 hips implanted with cobalt chromium molybdenum alloy-polyethylene porous surface replacements (PSR, Depuy, USA) were selected from the archive of tissues. Unfortunately, it was not possible to match the groups for time in situ or causes of failure. Furthermore, these had been processed for a previous study using slightly different staining techniques that were modified later. However, in a pilot study, a comparison of the two methods yielded essentially identical results (data not presented here).

The tissues were placed in optimum cutting temperature medium (OTC, Miles, Elkhardt, IN), frozen in liquid nitrogen and stored at –70 °C, pending processing. Serial sections were cut 6 μm thick and mounted onto positively charged glass slides. One section of each case was stained with hematoxylin and eosin to assess the general histomorphometric features of the tissue. We used a simple rating method to describe the number of macrophages, giant cells, lymphocytes, and wear particles, as well as the extent of fibrosis and necrosis (Doorn et al. 1996). Total macrophages and total T lymphocytes were stained using markers for EMB11/CD68 and T11/CD3, respectively (Dako, Carpinteria, CA).

Metal-on-metal tissues

Immunohistochemistry. Frozen sections were fixed for at least 12 hours at –20 °C in absolute acetone, then incubated for 30 minutes at room temperature with mouse anti-human monoclonal antibodies diluted in human serum diluent. After washing for 5 minutes in phosphate buffered saline, the sections were incubated with a biotinylated goat anti-mouse secondary antibody for 30 minutes at room temperature. The sections were again washed for 5 minutes in phosphate buffered saline and then incubated with Vectastain (Vector Labs Inc., Burlingame, CA) for 30 minutes at room temperature. Vectastain was conjugated with peroxidase enzyme and bound specifically to the biotin of the secondary antibody. The tissues were washed to remove unbound Vectastain; diaminobenzidine tetrahydrochloride was then used to react with the peroxidase conjugate for color visualization. The sections were counterstained with Gills hematoxylin (Sigma), dehydrated and mounted. Sections of fascia served as negative controls and spleen and tonsil were included in the staining to serve as positive controls. In addition, sections were stained after omission of the primary antibody, or with human serum diluent as further negative controls.

In situ hybridization. In situ hybridization was used to detect the presence of interleukin 1 beta (IL-1b), platelet-derived growth factor alpha (PDGF-α), interleukin 6 (IL-6), transforming growth factor beta (TGF-β), and tumor necrosis factor alpha (TNF-α). Messenger RNA sequences were determined from DNA sequences of the various cytokines used (GenBank, Los Alamos NM). Selected sequences were commercially synthesized (Operon, Alameda, CA) and biotinylated at the 3' end using biotin-11-dUTP and DNA deoxynucleotidylexotransferase (Gibco, Gaithersberg, MD). Prelabeled biotinylated probes were also obtained for some sequences (Genetics Research, Huntsville, AL). Poly-thymidine was used as a positive control and the hybridization solutions without labeled probe and labeled sense probes were used to assess the signal due to endogenous peroxidase and to act as a negative control respectively. We used probes of a uniform length (30 base pairs), with a guanine + cytosine: adenosine + thymine ratio of 0.6–0.7, and the method of tissue processing was standardized for all cases.

The protocol for in situ hybridization proceeded as for immunohistochemistry until the application of the working hybridization solution was applied. In this group, the solution was allowed to incubate at 42 °C overnight. The stained slides were washed

in 5×, 2×, 1× and 0.5× saline sodium citrate. Then a blocking solution of 10 mL 0.1M Tris/0.15M NaCl/5mM MgCl2, 200 μL normal sheep serum and 30 μL Triton-X, pH 7.5, was applied at room temperature for 1 hour, 2× anti-digoxygenin-alkaline phosphatase (Boehringer Mannheim, Germany) in the blocking solution was added (1: 200) and incubated for 1 hour at room temperature. The slides were washed twice in Tris-saline, at pH 7.5 and then pH 9.5. The colored precipitate was visualized with McGadey's solution (bromo-chloro-indoyl-phosphate and nitroblue tetrazolium in Tris/NaCl/MgCl2, pH 9.5). The slides were then dehydrated and mounted. Positive and negative controls were made.

Metal-polyethylene tissues

Immunohistochemistry. Frozen sections were fixed for at least 12 hours at –20 °C in absolute acetone and then incubated at room temperature with monoclonal mouse primary antibodies diluted in human serum diluent for 1 hour. The slides were washed for 20 minutes in phosphate buffered saline and incubated sequentially with rabbit anti-mouse immunoglobulins (RAM, Dakopatts a/s Denmark Z109), swine anti-rabbit immunoglobulins (SAR, Dakopatts a/s Z196) and soluble complexes of horseradish peroxidase and rabbit anti-horseradish peroxidase (PAP, Dakopatts a/s Z113) for 15 minutes each with a 20-minute buffer wash in between changes. Positively stained cells were visualized with 10 mg 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma, St. Louis, MO), 0.01% hydrogen peroxide, and 0.3% sodium azide in 0.05 Tris buffer, pH 7.6. The slides were counterstained with Gills hematoxylin (Sigma), dehydrated and mounted. Sections stained only with human serum diluent served as negative controls.

In situ hybridization. Frozen sections 6 μm thick were fixed for 10 minutes in 4% paraformaldehyde in phosphate buffered saline. pH 7.8, at room temperature. They were then washed in three changes of phosphate buffered saline/10% ethanol, dehydrated in 95% ethanol and absolute ethanol and air-dried for 30 minutes. The working hybridization solutions were prepared with probe concentrations between 0.5 and 2.0 ng/μL and incubated at 42 °C for 1 hour. The stained slides were washed in 5X saline sodium citrate and 2X saline sodium citrate. Streptavidin-peroxidase (Dako, Carpinteria, CA) in 0.1 Tris saline-Brij, pH 7.5 (1:200), was added and incubation continued for 1 hour at room temperature. The slides were washed in Tris saline, pH 7.5, for 10 minutes and diaminobenzidine hydrogen peroxide was used to visualize the positive areas. Gills hematoxylin number 3 was used as a counterstain.

Histological examination. Hematoxylin and eosin-stained frozen sections were examined to study the general histomorphometric features of the tissues. We used polarized light was used to detect wear particles, using the criteria of Willert et al. (1996)—i.e., birefringent particles were assumed to be ultra high molecular weight polyethylene and dense black particles, that have thin refractive edges when viewed with polarized light, were assumed to be metal particles. A semi-quantitative rating method, modified from that used by Mirra et al. (1976), was used to classify the numbers of macrophages, lymphocytes, giant cells and metallic wear particles from 0 to 3+ in nine fields of each tissue, using a 10×10 counting grid at 400× magnification (Doorn et al. 1996). The amounts of necrosis, fibrous tissue and polyethylene were noted, using a single score for the overall quality of the tissue sample.

Quantitative analysis

We counted 9 fields from each immunohistochemically stained tissue section, using a 10×10 counting grid at 400× magnification. About 50–300 cells were classified in each field for a total of 450–2700 cells per section. The percentage of positively-stained brown cells in the total number of cells was calculated. In cases where more than one tissue sample was available, the results were averaged. For in situ hybridization, the sections were examined with a computerized imaging program (Image 1.41, Macintosh) at a magnification of 200×. Since counterstaining was not done in the metal-on-metal group, only positive cells were counted in both groups. The total number of positive cells for each probe was counted.

We used a non-parametric Mann-Whitney test to determine if there were differences between the two groups.

Table 2. Results using semi-quantitative rating of general histological findings

Patient	Macro-phages	Giant cells	Metal particles	Lympho-cytes	Necrosis	Fibrous tissue	PE particles
1	+2	0	+1	+2	+1	+2	0
2	+3	0	+1	+2	+1	+1	0
3	+3	+1	+2	+2	+2	+1	0
4	+2	+1	+2	+2	+2	+2	0
5	+2	+1	+2	+2	+2	+1	0
6	+3	+1	+2	+1	+1	+1	+1
7	+2	+1	+2	+1	+3	+1	+1
8	+2	0	+1	+1	+2	+2	+1
9	+3	+1	+1	+1	+3	+2	+1
10	+3	+2	+1	+1	+2	+1	+1

Rating of cells and tissues: 1 minimal, 2 moderate, 3 marked

Table 3. Results of immunohistochemical staining and in situ hybridization positive cell counts

Patient	DIL	CD 3	CD 68	Hyb	IL-1β	IL-6	PDGF-α	TNF-α	TGF-β
Metal-on-metal									
1	0	35	36	0	220	1982	551	1324	725
2	0	10	28	0	1945	3822	2900	1807	662
3	0	19	33	0	1627	2429	2450	1719	1382
4	0	17	13	0	827	1064	1195	889	314
5	0	7	9	0	1079	1301	1360	632	832
Average	0	18	24	0	1140	2120	1691	1274	783
SD	0	11	12	0	677	1095	961	511	387
SE	0	5	5	0	303	490	430	229	173
Metal-on-PE									
1	0	10	77	0	1189	2237	1206	1941	1637
2	0	5	77	0	2739	3572	2478	3063	2284
3	0	4	36	0	479	798	372	1239	963
4	0	5	91	0	2792	4284	2763	3665	4003
5	0	11	97	0	2125	2843	1470	2623	2852
Average	0	7	76	0	1865	2747	1658	2506	2348
SD	0	3	24	0	1009	1333	973	948	1164
SE	0	1	11	0	451	596	435	424	521
P-values									
t-test		0.07	0.003		0.2	0.4	0.9	0.03	0.02
Mann-Whitney		0.06	0.01		0.2	0.5	0.9	0.05	0.02

SD standard deviation, SE standard error of mean

Results

General histomorphology (Table 2)

The sections from the two groups were similar as regards most of the histological features examined. The tissues usually showed a vascularized collagenous scar, with various numbers of macrophages, fibroblasts, giant cells and blood vessels. Several small zones of necrosis were seen in the M-PE group. Wear particles of birefringent UHMWPE were present in some of the cells in the M-PE group, while cells in the M-M group sometimes contained visible metal particles giving them a dusty appearance. Lymphocytes were noted in both groups, arranged perivascularly or in groups below the tissue surface.

Immunohistochemistry (Table 3)

Smaller numbers of CD68-stained macrophages were present in the M-M group tissues than in the

M-PE group. However, there were more T lymphocytes in the M-M tissues. These were diffusely distributed and, to a lesser extent, perivascular.

In situ hybridization (Figure, Table 3)

Cytokine staining was seen in all tissue sections. We found more cells that stained positive for TNF-α and TGF-β in the M-PE group than in the M-M group.

Discussion

Metal-on-metal hip replacements were reintroduced because of wear debris-induced osteolysis around failed M-PE total hips. Polyethylene wear particles are known to induce a florid macrophage and giant cell foreign body reaction that is associated with granuloma formation and the production of inflammatory and osteoclast-stimulating mediators. However, less is known about the tissue response to metal wear particles from M-M total hips, particularly regarding cytokine production. Doorn et al. (1996), in a histological study of periprosthetic tissues from several designs of M-M total hips, reported that the extent of the inflammatory reaction and the presence of foreign body type giant cells were much less marked than in tissues from metal-on-polyethylene components. Our findings accord with this observation, as fewer CD68-stained macrophages were present in the M-M group.

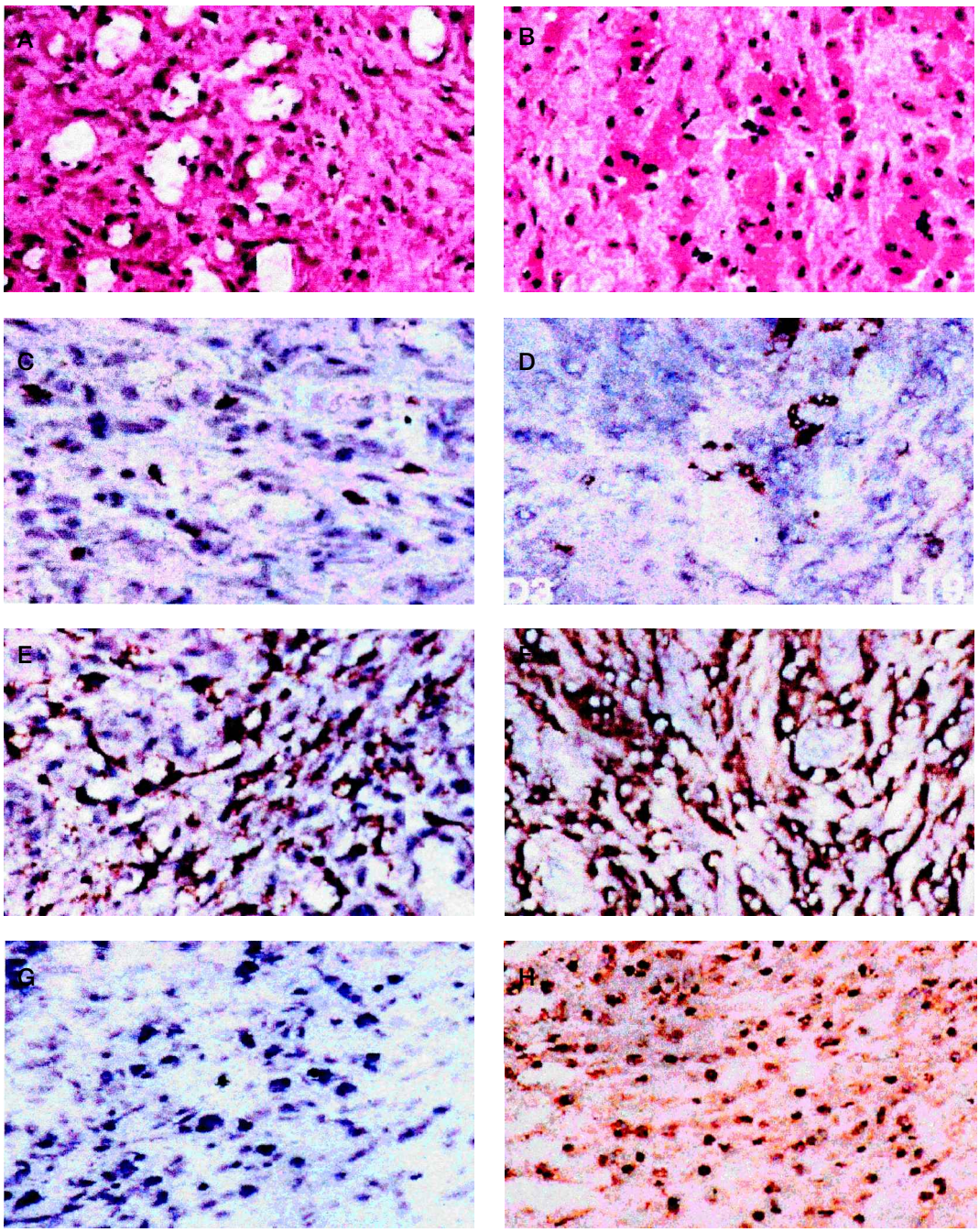
These authors also reported that wear particles from M-M hip bearings are an order of magnitude smaller than polyethylene particles and postulated that the lower tissue reactivity was due to the overall smaller size of metal wear particles. This hypothesis is consistent with the critical size range for biological reactivity to particles postulated by Green et al. (1998), namely 0.2–10 microns. Most of the metal wear particles analyzed by Doorn et al. are below this range, but most polyethylene particles are within it (Shanbhag et al. 1994).

In our study, M-M group tissues produced cytokines similar to those from M-PE tissues, presumably in response to wear particles. The semi-quantitative rating of macrophages in the M-M group was mostly 2+, indicating that visible particles were present intracellularly. This means that at least some proportion of the metal wear particles were within the critical size range and therefore might activate macrophages and fibroblasts to produce cytokines. Giant cells, however, were rarely seen in the M-M group, which is consistent with the smaller range in the size of the metal particles compared to polyethylene particles.

In contrast, there was a tendency to more lymphocytes in the M-M group. Willert et al. (2000) recently reported that tissues from 14 failed modern M-M total hips contained perivascular lymphocyte accumulations, sometimes in the form of follicles with germinal centers. Such features were absent in tissues from M-PE hip replacements. The authors suggested that this indicated of a delayed type hypersensitivity response to the metal wear products. Such responses have been reported in a few patients with metal implants made of alloys containing nickel (Merritt and Rodrigo 1996). The clinical significance of an increase in lymphocytes is not clear, as the 14 cases were revised for reasons apparently unrelated to a hypersensitivity response. However, among 12 failed Metasul M-M THRs examined by one of us (PC), one case was revised as a result of a suspected allergic response and acculumations of lymphocytes were present in the tissues.

The presence of high levels of TNF-a staining in the M-PE group accords with numerous reports of cytokine production around failed M-PE hips. (Chiba et al. 1994, Goodman et al. 1996). The production of cytokines around failed M-M total hips is less frequently reported even after more than a decade of clinical use of the Metasul components in Europe. The positive cytokine staining in these M-M surface replacement tissues suggests that the tissues around M-M total hips can induce bone loss. These results may seem at odds with the greatly reduced volumetric wear of M-M total hips. However, wear particles from the bearings are only one source, as particles may be produced by loose implants or third body wear secondary to mechanical damage, interface degradation or bone fracture. The importance of these particles to the process of cytokine production and osteoclast stimulation should not be overlooked, especially in total hips that use alternative bearings.

In summary, the results show that the cytokine profiles of M-M total hips are similar to those of



Representative findings in M-M vs. M-PE tissues.
A. HE in Case 2 (M-M)
B. HE in Case 10 (M-PE)
C. CD3 in Case 2 (M-M)
D. CD3 in Case 10 (M-PE)
E. CD68 in Case 2 (M-M)
F. CD68 in Case 10 (M-PE)
G. IL-6 in Case 2 (M-M)
H. IL-6 in Case 10 (M-PE)

M-PE hips and therefore warrant further study. An understanding of the factors that control the regulation of these inflammatory cytokines should increase the likelihood that alternative bearings will obviate the problems of wear debris that have arisen in the past.

The authors thank Phil Huie and Dr. Yong Song for their technical expertise.

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