RESORPTION OF BONE BY INFLAMMATORY CELLS DERIVED FROM THE JOINT CAPSULE OF HIP ARTHROPLASTIES

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The role of inflammatory cells in aseptic loosening and failure of cemented joint replacements is unclear. Inflammatory cells from the revision joint capsule of four failed hip arthroplasties were examined to determine their nature and resorptive capacity. The capsules contained numerous macrophages and abundant foreign-body macrophage polykaryons, distinguished from osteoclasts by their antigenic phenotype and lack of response to calcitonin.

When cultured on cortical bone slices in vitro, both macrophages and macrophage polykaryons produced small resorption pits and were associated with areas of superficial resorption of the bone surface. These results indicate that foreign-body induced macrophages and macrophage polykaryons are capable of a type of low-grade bone resorption which may be of pathogenic significance in the loosening of cemented joint prosthetic components.

Aseptic loosening is the commonest cause of late failure of cemented primary and cemented revision total joint replacements (Bergström et al 1973; Beckenbaugh and Ilstrup 1978; Charnley 1979; Carlsson and Gentz 1980). Following fixation of joint prostheses to bone, using polymethylmethacrylate (PMMA) cement, there is at first some necrosis of bone and bone marrow followed by repair with production of a fibrous tissue membrane at the cement–bone interface (Vernon-Roberts and Freeman 1976; Revell 1982). Inflammatory cells, including macrophages and giant cells, may form part of a cellular reaction to the PMMA and may contribute to loosening of the prosthesis by the process of bone resorption (Freeman, Bradley and Revell 1982; Johanson et al 1987).

The precise nature of the cell types found at the cement–bone interface is uncertain. In particular, it is not known whether the multinucleated cells found in this location are osteoclasts or foreign-body macrophage polykaryons (foreign-body giant cells, FBGC). The role of FBGCs and of other, mononuclear, inflammatory cells in bone resorption remains uncertain (Teitelbaum, Stewart and Kahn 1979; Mundy 1983). There is in vitro evidence that macrophage polykaryons of this type are not capable of bone resorption (Chambers and Horton 1984; Pazzaglia and Pringle 1988, 1989). However, several recent studies have shown that giant cells derived from giant-cell lesions of soft tissue are capable of bone resorption (Athanasou et al 1989; Flanagan and Chambers 1989). These polykaryons do not express an osteoclastic antigenic phenotype and do not respond to calcitonin. They have an ultrastructure distinct from the osteoclast but are capable of producing resorption pits when cultured on cortical bone slices (Athanasou et al 1989).

Our present study aimed to determine the antigenic and functional phenotype of the inflammatory cells present in the artificial joint capsule and synovial membrane surrounding a revision hip arthroplasty; this tissue contains identical wear debris components and a similar inflammatory cell response to that found in the fibrous tissue membrane at the cement–bone interface. In addition, since there is no bone in the capsule, there is no possibility of contamination by osteoclasts. We could therefore determine whether inflammatory cells responding to cement and wear debris are capable of bone resorption, a factor which could contribute to the loosening of cemented joint prostheses.

MATERIALS AND METHODS

Material consisted of the fibrous capsule and synovial membrane from four hip replacements undergoing revision arthroplasty (revision joint capsule). Specimens were received fresh in normal saline and radiographed to ensure that there was no calcified material in the capsule.
One block of the joint capsule was used for routine histology to exclude the presence of infection. A second block was taken for immunohistochemical studies, snap-frozen in liquid nitrogen and stored at -70°C prior to cryostat sectioning and immunostaining with monoclonal antibodies (Table I), using an indirect immunoperoxidase technique (Gatter, Falini and Mason 1984). The remainder of the specimen was used for extraction of inflammatory cells for in vitro culture.

**Preparation of cell cultures.** Culture medium RPMI/1640 (Flow, England) was supplemented with benzyl penicillin 100 IU/ml (Glaxo, England) and streptomycin 100 μg/ml (Glaxo, England) for cell isolation and 10% heat inactivated FCS (Gibco, England) (RPMI/FCS) for subsequent incubations. Salmon calcitonin (CT) was donated by Armour Pharmaceuticals, Eastbourne, England (4450 IU/mg) and dissolved (1 mg/ml) in 0.05% NaCl and 0.2% sodium acetate in distilled water containing 1 mg/ml of BSA. Hanks balanced salt solution (HBSS; GIBCO, England) was used for cell isolation.

The joint capsule was cut into small pieces and rinsed thoroughly in HBSS. The capsule fragments were digested for one hour at 37°C in HBSS containing 1 mg/ml collagenase type I (Sigma). The supernatant was decanted and centrifuged at 1500 rpm for ten minutes. The pellet was washed twice in HBSS and then resuspended in RPMI/FCS.

The suspension was added to pre-wetted bone slices, prepared as previously described (Athanassou et al 1989), and to 15 mm glass coverslips in 16 mm diameter Multiwell plates (Costar, England).

The cell suspension was incubated on these for two hours at 37°C then the bone slices and coverslips were removed, washed vigorously in RPMI and placed in fresh 16 mm diameter wells. Six bones were placed in wells containing 1 ml of RPMI/FCS. These were incubated for periods of four days and 11 days then two bones were fixed in 4% glutaraldehyde in 0.2 M cacodylate buffer for two hours. Two bone slices were placed in Triton-X-100 (0.1% in distilled water) for six hours before glutaraldehyde fixation. Triton treatment removes the cells from the bone surface and enables the underlying substrate to be examined and the number of resorption pits to be accurately counted. The specimens were dehydrated through a graded ethanol series and critical point dried from CO2.

Specimens were coated with gold and examined in a Philips SEM 505 scanning electron microscope. The number of giant cells on the bone slices and the number of resorption pits on the corresponding Triton-treated bone slices which had shared the same well were counted in cultures incubated for four and 11 days.

The cells cultured on coverslips, prepared as described above, were incubated in RPMI/FCS for 24 hours, four and 11 days in order to assess survival, number and antigenic phenotype of the giant cells and mononuclear cells over these periods. After fixation in
cold acetone, immunocytochemistry was performed on
the coverslips using EBM/11, a monoclonal antibody
that strongly stains macrophages and macrophage poly-
karyons.

**Response to calcitonin.** Following digestion, the cell
suspension was added to the wells of a 16 mm diameter
Costar plate containing a 15 mm glass coverslip; these
were incubated for 20 minutes at 37°C in 5% CO₂. The
coverslips were then removed from these wells and
washed vigorously to remove non-adherent cells. One of
a pair of coverslips was then placed in a well containing
CT (2 IU/ml), the other in control tissue culture medium
(FCS/RPMI). The cells were incubated and continuously
observed for up to one hour, after which they were fixed
in formalin for Giemsa staining. This was repeated using
cells incubated on pairs of coverslips for 24 hours in FCS/
RPMI.

**RESULTS**

**Histology and immunohistochemistry.** The revision joint
capsule contained abundant histiocytes and foreign-body
giant cells in relation to foreign material (acrylic cement
and high density polyethylene). Some fibrous capsules, at
least in part, had a hyperplastic synovial intima-like
lining, but for the most part there was no distinct synovial
lining, the surface being covered by inflamed fibrous
tissue containing histiocytes and giant cells. No calcified
material was present in the revision capsules studied.

Evidence that the majority of the mononuclear cells
present in the revision capsule were macrophages was
provided by immunohistochemical staining (Table I). These
cells showed strong membrane staining for CD11a,b,c (LFA-1 family), CD13, CD14, CD16 (FcRII), CD18, CD32 (FcRII), CD35 (CR1),
CD45 (leucocyte common antigen), HLA-DR, CD68
(macrophage-associated antigen) (Fig. 1). Macrophage
polykaryons showed an identical pattern of immunoreac-
tivity. Expression of such a wide range of macrophage
markers by the giant cells is characteristic for macrophage
polykaryons and is distinct from that of osteoclasts which
express a highly restricted set of leucocyte and macro-
phage-associated antigens (Athanassou and Quinn 1990).
Hyperplastic synovial lining cells which were present on
the inner surface of the membrane showed an identical
pattern of immunoreactivity to that of mononuclear
inflammatory cells in the membrane.

Both mononuclear cells and polykaryons were
derived from the revision joint capsules which were
incubated for four and 11 days on glass coverslips. Both
mononuclear and multinucleated cells reacted strongly
with the macrophage marker EBM/11 (anti-CD68).
Greater numbers of polykaryons were seen in 11-day as
compared with four-day cultures.

**Response to calcitonin.** Giant cells settled onto glass
coverslips were observed by phase contrast microscopy.
After settling, the cells quickly expanded their cytoplasm
to produce a broad cell outline with broad pseudopodia.
After salmon calcitonin 2 IU/ml was added to the culture...
medium, polykaryons were observed up to two hours, and no change in the motility or shape of the cells in response to calcitonin was observed. Osteoclasts show a highly specific and rapid response to calcitonin which induces immotility and retraction of cytoplasmic processes (Chambers and Magnus 1982).

**Cell cultures on bone slices and SEM bone resorption assay.** Cell culture on bone slices showed numerous mononuclear cells of macrophage or fibroblast morphology and ten to 30 scattered cells of large size (up to 150 μm) (Figs 2a to 2c). The large cells had a few scattered microvilli and ruffles over their free (upper) surface. Some large cells had extended lobulated or folded pseudopods but most were rounded and flattened against the bone surface. Small widely spaced retraction fibres at the cell margin bound the cells tightly to the bone surface. In several cultures on bone slices, a few osteoclast-like resorption pits were noted on the bone surface (Table II). These were well-defined excavations with a coarsely fibrillar base of mineralised collagen fibres (Figs 2d, e). These resorption pits were generally circular or ovoid in outline and were most often small and single (Fig. 2d) or in small groups of tiny pits, often associated with a nearby area of surface resorption. Relative to the large number of multinucleated cells and mononuclear cells...
present on the bone slice, very few resorption pits were formed by these cells.

A second common type of bone resorption was also noted in the vicinity of both mononuclear and multinucleated cells on all bone slices. These were poorly defined large and small areas of roughening or resorption of the bone surface with exposure of mineralised collagen fibres (Figs 2a,b). These areas of surface roughening or resorption were seen beneath both mononuclear and multinucleated cells, most often in the vicinity of retraction fibres at the margin of the cell. Surface roughening was most common around groups of macrophages and multinucleate cells and appeared more pronounced after cell culture on bone slices for 11 days compared with four days.

DISCUSSION

This study has shown that there are cells in the revision joint capsule which are capable of bone resorption. The major cell types identified in the inflammatory infiltrate in the capsule were histiocytes and foreign-body macrophage polykaryons. These cells produced a different degree and pattern of bone resorption compared to that known to be associated with osteoclasts. Very few resorption pits, generally of small diameter and area, were produced, and there was considerable evidence of surface roughening or resorption of the bone surface. These features indicate that histiocytes and foreign-body giant cells are capable of bone resorption. They also suggest that such low-grade bone resorption may occur in other locations where histiocytes and macrophage polykaryons respond to identical wear debris, such as the interface between the bone and implant in total hip replacement.

The revision joint capsule which forms in relation to the prosthesis is composed largely of fibrous tissue and has an inner lining resembling normal synovium with a layer of synovial lining cells. Reaction to particulate debris is seen within this capsule and is largely in the form of a histiocytic and macrophage polykaryon response to metal, polyethylene and PMMA (Vernon-Roberts and Freeman 1976; Revell 1982). In general, metal debris is not often seen where metal articulates with polyethylene, as in the arthroplasties revised in this study, where the most commonly seen foreign materials were polyethylene and acrylic bone cement. Foreign-body macrophage polykaryons were seen in relation to this foreign material histologically, and their identity and distinction from osteoclasts were confirmed by their failure to respond to calcitonin and their antigenic phenotype. Osteoclasts rapidly and specifically respond to calcitonin administration by becoming immotile and retracting their cytoplasmic processes (Chambers and Magnus 1982). Foreign-body giant cells in the revision joint capsule have an antigenic phenotype which closely resembles that of monocytes and macrophages whilst osteoclasts express a highly restricted range of monocyte/macrophage antigens (namely, CD13, CD31, CD44, CD45, CD54 and CD68) (Athanassou and Quinn 1990). In particular, macrophage polykaryons, unlike osteoclasts, are known to express the LFA family of antigens (CD11a,b,c and CD18), CD14, HLA-DR, several Fc receptors (CD16, CD32, CD64), and receptors for complement components (CD11b,c, CD35). Mononuclear macrophages, which were also abundant in the inflammatory infiltrate in the revision joint capsule, showed an identical pattern and intensity of staining, as did the hyperplastic lining cells covering the inner surface of the joint capsule. No bone particles or calcified material were seen on specimen radiography or histologically so it would appear that the cells producing the few resorption pits and surface resorption on bone slices were not osteoclasts but either inflammatory cells or synovial lining cells, both of which are macrophage marker positive.

Although it is generally believed that osteoclasts alone are capable of bone resorption, several recent studies have shown that giant cells, derived from pathological lesions in extra-osseous locations, are also capable of bone resorption (Athanassou et al 1989; Flanagan and Chambers 1989). These giant cells, like the macrophage polykaryons isolated from revision joint capsules, do not respond to calcitonin and exhibit an antigenic phenotype which closely resembles that of tissue macrophages. Bone resorption by tumour-associated macrophage polykaryons was also associated with very few resorption pits of small diameter and volume and areas of surface roughening. This pattern contrasts markedly with that of osteoclastic bone resorption where numerous resorption pits, many of which are often huge in area or volume, are found on cortical bone slices (Chambers et al 1984, 1987).

Given the distinguishing structural, functional and immunophenotypic differences between osteoclasts and macrophage polykaryons, this difference in the pattern of bone resorption is hardly surprising. However, the fact that macrophage polykaryons and mononuclear phagocytes are capable of a low-grade type of bone resorption has considerable implications for investigations into osteoclast ontogeny and function, and for the study of pathological bone resorption associated with tumours and inflammation. One of the functional criteria by which a polykaryon is defined as an osteoclast is its ability to produce bone resorption on cortical bone slices.

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<th>Table II. The number of pits and area resorbed per bone slice by foreign-body giant cells</th>
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<td>Number of giant cells</td>
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In the light of our findings, this operational criterion would now appear to be insufficient; it should be supplemented by other defining characteristics such as ultrastructural features on transmission electron microscopy, functional response to calcitonin and immunophenotypic analysis. This is particularly relevant for cells produced in long-term marrow or spleen cultures which have also shown a type of low-grade bone resorption (Kukita et al 1989).

The pathological significance of low-grade osteolysis associated with the macrophage polykaryon and histiocytic response to wear debris is relevant to loosening of prostheses. Factors which may contribute to loosening include purely mechanical or technical factors such as insufficient contact between bone and cement due to faulty technique at implantation, faulty position of the implant, or high friction between the components of the prosthesis (Vernon-Roberts and Freeman 1976; Revell 1982). Abnormal mechanical stresses on the artificial joint such as excessive physical activity or increased body-weight are also likely to be important as is the development of infection related to the prosthesis (Vernon-Roberts and Freeman 1976; Revell 1982). Loosening of the prosthesis results in production of significant wear debris to which there is often a noteworthy macrophage and giant-cell reaction; this can extend into bone related to the implant (Vernon-Roberts and Freeman 1976; Freeman et al 1982; Revell 1982; Johanson et al 1987). In the fibrous membrane between bone and cement and in the bone-marrow spaces adjacent to the implant, there may also be an associated histiocytic and giant-cell response to wear debris.

The low-grade bone resorption exhibited by macrophage polykaryons and mononuclear phagocytes found in relation to wear debris may be a significant contributory factor to loosening of the prosthesis. The fact that such osteolysis is not as dramatic as that associated with osteoclasts also accords well with the known clinical pattern of loosening of prosthetic implants.

Osteoclastic bone resorption associated with necrosis of bone is occasionally seen in relation to some implants and may contribute to accelerated loosening of the prosthesis (Vernon-Roberts and Freeman 1976; Revell 1982). In addition, as suggested by several observers, stimuli known to excite macrophages, such as products of cell death, bacteria and foreign particulate matter, would also contribute to increased bone resorption by the histiocytes and macrophage polykaryons in the fibrous membrane (Vernon-Roberts and Freeman 1976; Johanson et al 1987). More precise identification of the factors controlling such macrophage and macrophage polykaryon mediated low-grade bone resorption clearly merit further study.

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REFERENCES


