IMMUNOPATHOLOGICAL RESPONSE TO LOOSE CEMENTLESS ACETABULAR COMPONENTS

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The membranes surrounding seven loose cementless acetabular implants were shown to contain polyethylene particles, birefringent in polarised light. Three of these implants were made of titanium alloy and the membranes around these contained titanium particles as well. There was no metallosis around the four implants made of chromium-cobalt-steel alloy.

Both titanium and polyethylene particles caused migration, adherence and phagocytosis of CD11b-positive, peroxidase-negative macrophages. There were no histological signs of activation of the specific immune response; neither interleukin-2 receptor-positive activated T cells nor PCA-1 plasmablasts/plasma cells were present in the tissues.

In cases of simple loosening, resident mesenchymal fibroblast-like cells were active. In aggressive granulomatosis, there were many macrophages and multinucleated giant cells but little fibroblast reaction.

The clinical relevance of the findings is that the use of cementless prostheses is not a guarantee against adverse tissue reactions.

Previous research has shown that loosening of cemented total hip replacements is sometimes caused by adverse tissue reactions which have a peculiar immunopathological character (Harris et al 1976; Tallroth et al 1989; Santavirta et al 1990b). This is one of the reasons for the current interest in cementless prostheses (Eskola et al 1990).

Some cementless prostheses which were originally well implanted loosen unpredictably from adverse tissue reactions around the acetabular component. We recently showed that this may be due to an aggressive granulomatous reaction, possibly caused by polyethylene debris (Santavirta et al 1990a).

We now describe the immunopathological response around loose cementless acetabular components.

MATERIALS AND METHODS

Seven patients had revision operations for painful loosening of cementless acetabular components (Table I). There were four men and three women, and at the time of the revision their average age was 52 years (39 to 67). The primary hip condition which led to the cementless total hip replacement was primary osteoarthritis in two cases, secondary osteoarthritis after femoral neck fracture in one, congenital luxation in one, and old epiphyseolysis of the femoral head in three. Chrome-cobalt-steel alloy prostheses (Lord) had been used in four patients and titanium prostheses (Biomet) in three. The revision operations were performed an average of 4.8 years (1.3 to 7.5) after the arthroplasty. The patients had suffered pain for an average of 1.4 years (0.7 to 2.0).

At revision, four patients had simple loosening with a synovium-like layer of tissue around the acetabular component. Three patients had aggressive granulomatosis with large cysts containing granulomatous tissue beneath the acetabular cup. All three patients with titanium prostheses, one with aggressive granulomatosis and two with simple loosening, showed metallosis at revision.

At the revision operation, the granulomatous or membranous tissue layer was removed, and specimens were examined immunohistopathologically. Bacterial cultures were sterile in them all. All the patients had
normal blood sedimentation rates and C-reactive protein levels.

Biopsy samples, 3 to 4 mm in diameter, were embedded and frozen in OCT compound (Lab-Tek Products, Naperville, Illinois). The immunostaining was done on 6 μm thick cryostat sections (Konttinen et al 1983a). They were fixed in cold (+4°C) acetone for five minutes and the endogenous peroxidase activity was blocked with 0.3% H2O2 for 15 minutes. Then the sections were treated serially with normal horse serum (1:5) for 20 minutes, and with appropriate concentrations (Konttinen et al 1983b) of mouse monoclonal antibodies OKT11 (= CD2 for total T cells), OKT4 (= CD4 for inducer/helper T cells), OKT8 (= CD8 for suppressor/cytotoxic T cells), OKM1 (= CD11b for monocytes and null cells), OKIa (for common framework epitope in major histocompatibility complex II antigen), or Tac (for interleukin-2 receptor) (Breard et al 1980). All the monoclonal antibodies of the OK series were from Ortho Diagnostic System Inc, New Jersey; the pan-B (= CD19 for resting B lymphocytes) was from Dakopatts, Copenhagen, the PCA-1 for plasmablasts and plasma cells came from Coulter Immunology, Florida, and the anti-PC, a monoclonal antibody for the carboxyterminal propeptide of type I collagen, was provided by John A. McDonald, Washington University School of Medicine, St Louis, Missouri. Each of these agents was applied for 60 minutes. Each section was then exposed to biotinylated antimouse IgG for 30 minutes and avidin-biotin-peroxidase complex (ABC) for 30 minutes (Hsu, Rainie and Fanger 1981). The ABC kit was purchased from Vector Laboratories, Burlingame, California. Finally, the sites of peroxidase binding were revealed with 3,3’- diaminobenzidine tetrahydrochloride (Sigma Chemical Co, St Louis, Missouri). All the sections were counterstained with hematoxylin. Between each step, the slides were washed twice in phosphate-buffered saline (PBS, 0.1 M, pH 7.3). The above named antibodies were selected because they cover the cell subsets anticipated to be of interest.

The staining controls were used 1) omission of the primary monoclonal antibody in the staining sequence, and 2) the use of inappropriate mouse myeloma protein instead of a specific monoclonal antibody. In addition, histochemical staining of untreated (= no inhibition of endogenous peroxidase) tissue sections was done to reveal endogenous peroxidase-positive monocytes and granulocytes.

Biopsy samples 3 to 4 mm in diameter embedded and frozen as above were used for non-specific esterase staining (Konttinen et al 1983b). Cryostat sections 6 μm thick were prepared and fixed in acetone for five minutes at +4°C, and incubated in a medium consisting of 35 ml 0.067 mol/l PBS (pH 7.6), 2.4 ml hexazotised pararosanilin and 16 mg alpha-naphthyl acetate in 2 ml ethylene glycol monomethyl ether (the mixture was adjusted to pH 6.1 using 0.067 mol/l KH2PO4 or Na2HPO4). Incubation was done 0.5 to 2 hours at room temperature, and the sections were counterstained with 1% aqueous solution of toluidine blue.

The histological specimens were evaluated blindly. Peroxidase-positive cells showed a peripheral brown rim; non-specific esterase-positive cells displayed diffuse brown cytoplasmic staining.

The cells were counted from 6 μm tissue sections using an ocular counting square (20 × 20 squares) and an oil immersion objective (×1000 magnification). The numbers were expressed as a percentage of all the mononuclear cells in the microscopic field. At least 100 cells were counted for each differential count. The interobserver and intra-observer variations did not exceed 15%. The histopathological samples were examined with polarised light for birefringent particles (polyethylene).

**RESULTS**

Histologically the samples from patients who had simple loosening of the acetabular component were composed of dense connective tissue, with slender elongated fibroblasts embedded in a relatively dense extracellular
collagenous tissue matrix. The samples from patients who had aggressive granulomatosis consisted of fibroblasts and histiocytic-monocytic reactive zones. The three patients with titanium prostheses had metallosis of the extracellular matrix with fragments occasionally inside the macrophages.

In the inflammatory cell infiltrates, T lymphocytes were usually relatively few (Table II and Fig. 1). Among CD2-positive lymphocytes, the CD4-positive subset always formed the majority with a CD4/CD8 ratio of approximately two to one in all patients except case 6, in whom the ratio was approximately six to one. Furthermore, there was a total lack of interleukin-2 receptor-positive T lymphocytes, suggesting that the few T lymphocytes present were not activated. Practically all the local lymphocytes belonged to the thymus-dependent T cell series as shown by negative CD19 and PCA-1 staining for B lymphocytes and plasmablasts/plasma cells respectively.

The most frequent cell stainable with the extensive panel of monoclonal antibodies applied was the CD11b-positive tissue macrophage (Table II and Figs 2 and 3). These cells were mature tissue macrophages rather than recently recruited peripheral blood mononuclear cells as shown by the lack of histochemical staining for endogenous peroxidase. Endogenous peroxidase-positive polymorphonuclear neutrophil leucocytes were only occasionally seen in the intravascular compartment and not at all in the tissue itself, suggesting that such cells do not migrate to tissues in this type of reaction. Due to heavy peroxidase staining of CD11b-positive tissue macrophages, phagocytosed titanium and/or polyethylene particles were more easily discernible in slides stained for T cell epitopes (Fig. 1).

Finally, many of the local cells in the connective tissue matrix had a slender, fibroblast-like appearance (Fig. 4). Some of these cells were activated fibroblasts as shown by positive labelling for the carboxyterminal propeptide of interstitial type-I collagen (Table II and Fig. 4) found in the activated but not in the resting fibroblasts. Furthermore, this antiserum also stained cross-reactive material in the vascular endothelial cells.

**DISCUSSION**

Fixation of a prosthesis requires the development of a biological interface between the implant and the living bone and in well-fixed cases this membrane is thin and synovium-like (Gristina 1987). Around loose cementless acetabular components, particulate polyethylene can be demonstrated by polarised light microscopy (Lord et al 1988; Santavirta et al 1990a). These particles were usually surrounded by macrophages and, in the case of titanium prostheses, these cells contained metal fragments. Light microscopy showed monocyte/macrophage migration,
adherence and phagocytosis, and these reactions were particularly intense in cases of aggressive granulomatosis (Goldring et al 1983; Jasty et al 1986; Jones and Hungerford 1987). In our series metallosis was seen in all three cases with titanium prostheses and in none of the chromium-cobalt-steel alloy prostheses. However, the pathological significance of titanium metallosis is unclear since the aggressive granulomatous type of reaction occurred with both types of implant.

Our findings suggest that cells belonging to the mononuclear phagocyte lineage predominate in the response to loosening of cementless acetabular components. Bone-marrow-derived macrophage-like cells probably migrate into the reactive tissue, adhere to polyethylene and/or titanium particles and, if these are small enough, phagocytose such particles. The presence of macrophage accumulations, occasional multinucleate giant cells and sometimes granulomatous formations, all suggest a foreign-body reaction (Chambers 1978). However, the recruitment of the mononuclear phagocytes seems not to be very intense as evidenced by the rarity of freshly recruited endogenous peroxidase-positive peripheral blood monocytes.

Indigestible foreign metal or plastic particles may cause a continuous stimulus to the reactive mononuclear phagocytes, but this macrophage response does not of itself activate the lymphocyte-mediated immune response. Although foreign metal or plastic particles are only weak immunogens mesenchymal fibroblast-like cells can be activated in such a process.

The involvement of macrophage and fibroblast-like cells in the response to indigestible particulate material derived from implants is not unexpected since it is the role of macrophages to remove the debris of damaged tissue, and the role of the fibroblast is to lay down new tissue during healing (Konttinen et al 1989). However, our immunohistopathological analysis does not explain why simple loosening is characterised by fibroblast activation, whereas these cells are relatively rare in aggressive granulomatosis.

Titanium metallosis and the presence of scattered polyethylene particles was not restricted to cases of aggressive granulomatosis. Our previous work has shown
that aggressive granulomatosis occurs in both cemented and cementless total hip replacements (Tallroth et al 1989; Santavirta et al 1990a). It does not seem to be dependent on the chemical nature of the irritant, but rather represents a non-specific foreign-body reaction. The clinical relevance of this observation is that the use of cementless prostheses is not a guarantee against adverse tissue reactions.

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