Anti-inflammatory properties of titanium in the joint environment

AN EXPERIMENTAL STUDY IN RATS

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Little is known about the tissue reactions to various implant materials which coincide with an inflammatory reaction. We used the avridine arthritis rat model to evaluate the tissue response in the synovial, interstitial and subcutaneous tissues after implant insertion.

Quantitative immunohistochemistry showed that normal joint synovial tissue is dominated by ED2-positive resident macrophages. Polyethylene implants induced a much stronger foreign-body reaction than titanium implants, as measured by the number of interfacial ED1-positive macrophages. The tissue response to titanium and polyethylene was also vastly different in arthritic synovial tissue compared with control tissue.

It is likely that these biomaterials interact differently with inflammatory cells or intermediary compounds. It may be that arthritic synovial tissue produces reactive oxygen intermediates (free radicals) with which titanium has a unique anti-inflammatory interaction in vitro.


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Patients with rheumatoid arthritis develop progressive joint inflammation resulting in cartilage destruction and deformity of the joints. Loosening of prostheses is a particular problem for such patients making the choice of the material and design of an implant difficult. Experimental arthritis has been established in several species, but the rat arthritis model using adjuvants has some advantages. This species is large enough to allow insertion of implants in the knee and reagents are available to detect various cells and cytokines immunohistochemically. Little is known about tissue reactions to various implant materials which coincide with an inflammatory reaction, but macrophage infiltration is crucial in the inflammation seen in an implant-induced foreign-body reaction and in the destruction of rheumatoid joints.

Foreign implanted material elicits macrophage recruitment as well as producing a response in the resident macrophages. We used rat-specific ED1 and ED2 monoclonal antibodies to identify the different macrophage subpopulations. These have been shown not to react with other cell types or tissue structures. ED1-positive cells have previously been shown to be involved in the tissue response to implant materials whereas ED2-positive cells seem to be predominant in normal synovial tissue.

Both polyethylene and titanium are regularly used for implants and titanium has been suggested to have anti-inflammatory properties. We therefore compared these two materials with respect to the tissue reaction in normal synovial tissue and in adjuvant arthritis.

Materials and Methods

We used 40 male Lewis rats weighing 220 to 240 g (Moellegaard Breeding and Research Centre A/S, Lille Skensved, Denmark).

The induction of arthritis and the insertion of implants were done under pentobarbital anaesthesia. Arthritis was induced by the intradermal injection at the base of the tail of 150 μl of avridine (CP 20961 = N,N-dioctadecyl-N',N'-bis(2-hydroxyethyl) propanediamine; Pfizer Inc, Groton, Connecticut) solubilised in Freund’s incomplete adjuvant (50 mg/ml). The animals were given the analgesic buprenorphine 0.05 mg/kg three times a day, since in a pilot study we had found that animals not so treated developed muscular atrophy and feeding problems. Titanium or polyethylene implants were inserted in the knee, abdominal wall and subcutaneously on the back in eight arthritic and six normal rats. Six normal and six arthritic rats without implants were used as a control group. Four rats were excluded from the arthritis group; two died and two did not develop arthritis.

One normal rat was excluded because the specimens were of poor quality. This left 35 rats in the study.

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We used disc-shaped implants of non-alloyed titanium (Avesta AB; Avesta, Sweden) and polyethylene (Chirulene; Poly Hi Solidur, Vreden, Germany). The knee implant had a diameter of 3 mm and was 1 mm thick and that for the abdominal wall and subcutaneous tissue had a diameter of 5 mm and was 2 mm thick. The surface roughnesses were similar for the two implant materials. All the implants were rinsed in 70% ethanol in water and in distilled water in a sonic bath. The titanium implants were then autoclaved. The polyethylene implants were sterilised with ethylene oxide, followed by four cycles of vacuum and aeration at 40°C. The implants remained at room temperature for at least two weeks to ensure that all residues of ethylene oxide had been removed.

Through an anterolateral incision one implant was placed in the suprapatellar recess and the joint capsule closed with a non-resorbable monofilament suture. Another was placed in the abdominal wall between the peritoneum and the rectus muscle and a third in the subcutaneous tissue of the back. After 28 days, the animals were killed by an overdose of pentobarbital. The knee implants were found in the antero-medial part of the suprapatellar recess. The capsule with the adhering implant was retrieved en bloc. Control synovial tissue was taken from the same location. The specimens were cooled in saline on ice, mounted on cork, cryoprotected with Histocon (Histolab Products AB, Sweden) and snap frozen in isopentane cooled on dry ice.

The implants were then carefully removed from the frozen specimens with care to avoid thawing during this procedure. Many implants, about 20%, fell off or were easily removed before freezing. Sections 5 μm thick were cut in a cryostat (Bright Instrument Company, Huntingdon, UK), placed on alun-coated glass slides and allowed to air dry. The slides were kept at ~70°C until stained and then fixed in ice-cold acetone. Endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide in pure methanol. Unspecific binding was blocked by horse serum. Thereafter the slides were incubated for one hour with the primary monoclonal mouse anti-ration macrophage-specific antibody ED1 or ED2 (MCA 341 and MCA 342; Serotec Ltd, Oxford, UK) diluted 1:100. They were then incubated with affinity-purified and biotinylated horse anti-

Table 1. Median number (range) per mm² of ED1- and ED2-positive cells in and thickness (μm) at the tissue sites in arthritic and control groups with either polyethylene or titanium implants.

<table>
<thead>
<tr>
<th>No implant</th>
<th>Normal n</th>
<th>Arthritis n</th>
<th>Normal n</th>
<th>Arthritis n</th>
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<td>(80 to 3280)</td>
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<td>(0 to 480)</td>
<td>(4240 to 10 080)</td>
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<td>(1800 to 4640)</td>
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<td>(0 to 480)</td>
<td>(0 to 320)</td>
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<td>(0 to 480)</td>
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<td>3180</td>
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<tr>
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<td>(2600 to 4880)</td>
<td>(2000 to 4880)</td>
<td>(3600 to 4880)</td>
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<td>(50 to 300)</td>
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<tr>
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<td>3180</td>
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<td>5460</td>
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<td>(3200 to 4880)</td>
<td>(3200 to 4880)</td>
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</tbody>
</table>

* number of observations
† significantly different from normal synovial tissue without implant
‡ significantly different from respective synovial tissue without implant
§ significantly different from synovial tissue with titanium implant
†† significantly different from normal synovial tissue without implant
‡‡ significantly different from arthritic synovial tissue with titanium implant
††† significantly different from subcutaneous tissue with titanium implant
‡‡‡ significantly different from subcutaneous tissue without implant

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mouse IgG-specific antibodies followed by Vectastain ABC (Vector Laboratories Inc, Burlingame, California) for 30 minutes. Sections were developed for peroxidase activity using 3-amino-9-ethyl-carbazole (Sigma Chemical Co, St Louis, Missouri). The slides were counterstained in May-
er’s haematoxylin.

All specimens were assessed blind by light microscopy. For each specimen the cells within 50 μm from the implant in the interfacial zone and throughout the foreign-body capsule were counted using a 10×10 grid. Each square covered an area of 50×50 μm. The grid was aligned with the interface and we counted the cells within the closest row and also throughout a 500 μm thick segment of the joint capsule. Since the synovium and the implant-induced foreign-body capsule are difficult to distinguish from each other we have in this study used the distance from the synovial lining to the underlying muscle tissue as a general measure and named it synovial thickness irrespective of whether an implant is present or not. For the synovial specimens without implants we coated the cells within 50 μm from the synovial lining as well as throughout the capsule.

Statistical analysis. Differences between non-implanted arthritic and normal joints as well as between titanium and polyethylene implants in subcutaneous or interstitial tissues were evaluated using the Mann-Whitney test (MWT) for independent samples. Differences between joints implanted with titanium or polyethylene implants or without implants were analysed using the Kruskal-Wallis test (KWT). Post-hoc testing to detect individual group differences was done according to Siegel and Castellan.

Results

After two weeks 14 of the 16 rats injected with avridine developed clinical systemic arthritis with weight loss and joint swelling. The arthritis proliferated from the hindpaws to the knees and to the forelimbs. None of the rats showed signs of infection.

All the results are shown in Table I and the results from the synovial specimens also as box plots (Figs 1 and 2) and microphotographs (Fig. 3).

Box plot showing the ED1 cell density in arthritic and non-arthritic joints at the interface to titanium and polyethylene implants as well as in the normal synovial tissue. The box represents the 25 and 75 percentiles and the median value is indicated by the horizontal line within the box. a, significantly higher numbers of ED1-positive cells compared with all other situations; b, significantly higher numbers of ED1-positive cells compared with normal synovial tissue; c, significantly different from normal synovial tissue.

Box plot showing the ED2 cell density in arthritic and non-arthritic joints at the interface to titanium and polyethylene implants as well as in the normal synovial tissue. The box represents the 25 and 75 percentiles and the median value is indicated by the horizontal line within the box. a, significantly different from synovial tissue without implants and arthritic synovial tissue with titanium implants; b, significantly different compared with synovial tissue without implants.)

Synovial thickness. There was no significant difference (MWT) between non-implanted arthritic joints and normal joints. The presence of a titanium as well as a polyethylene implant significantly increased the synovial thickness (KWT) in the arthritic joints (p = 0.02 and p = 0.03, respectively) but not in the normal joints. There was no significant difference (KWT) between the polyethylene and titanium implants with respect to the thickness of the synovial capsule in either the normal or the arthritic joints.

Synovial cells in the interfacial area. In non-implanted joints there was a significant difference (MWT) between arthritic and non-arthritic joints in regard to the total numbers of cells. In normal joints most cells in the synovial lining were ED2-positive (Fig. 3b) whereas in arthritic joints there was an infiltration of ED1-positive cells reflected as a significant increase in ED1-positive cells in the interface zone (Fig. 3c; MWT, p = 0.0065). The ED1-positive cells at the interface were significantly higher in normal and arthritic joints with polyethylene implants compared with respective non-implanted joints (KWT, p = 0.0025 and p = 0.0055). In arthritic joints there was also a significantly higher number of ED1-positive interfacial cells at the polyethylene implant compared with those at the titanium implant (KWT, p = 0.028). The total numbers of cells were not significantly different when comparing the two materials in normal and arthritic joints (KWT). Polyethylene-implanted joints had significantly decreased numbers of interfacial ED2-positive cells compared with non-implanted non-arthritic (KWT, p = 0.044) and...
Fig. 3
Microphotographs of representative tissue sections. Panel a and b shows the normal synovial tissue stained for ED1- and ED2-positive cells respectively. Positive staining appears red-brown and the nuclei are blue. Panels c and d show the arthritic synovial tissue. Panels e and f show the arthritic synovial tissue with a titanium implant. Panels g and h show the arthritic synovial tissue with a polyethylene implant.
arthritic joints (KWT, p = 0.0043). There was no significant change in cellular composition and total cell density in arthritic joints with titanium implants compared with those without implants (KWT), but there was a clearly significant difference between polyethylene and titanium implants in arthritic joints in regard to ED1- (KWT, p = 0.028) and ED2-positive interfacial cells (KWT, p = 0.031).

Subcutaneous and interstitial implants in normal and arthritic rats. The cell density of ED1-positive interfacial cells was significantly higher for polyethylene implants in normal but not arthritic rats compared with titanium implants (MWT, p = 0.043) in the same situations. No other significant differences were seen between the two materials or between the arthritic or non-arthritic groups.

In the subcutaneous implants in arthritic rats, polyethylene gave significantly higher total cell densities (p = 0.0055) as well as numbers of ED1-positive cells in the interface compared with titanium implants (MWT, p = 0.0055). No significant differences between arthritic and non-arthritic rats were seen for the subcutaneous titanium implants.

Discussion

There are several problems associated with the use of implants in rheumatoid patients and it is not known how the presence of the artificial joint influences the inflammatory disease. Arthritic synovial tissue is known to contain an increased number of inflammatory cells such as macrophages, polymorphonuclear cells and T-lymphocytes and elevated levels of cytokines. These cell types are also seen at the site of implanted biomaterials and it is conceivable that the arthritic condition will alter the tissue response and the long-term outcome of the implantation of prostheses.

For the evaluation of biomaterials it is preferable to use longer implantation times and to insert the implant when the arthritis has already developed. Such modifications, however, are difficult to make considering the generalised disease of the animal and the need to implant the materials at the time of induction of the arthritis. The present findings are in accordance with the arthritic arthritis described by Vingbo et al. and classic adjuvant arthritis. The synovitis in the knees was mild and characterised primarily by the infiltration of immunocompetent cells, especially lymphocytes and macrophages. The knees did not show the severe changes with large clusters of neutrophil cells, new periosteal bone formation and cartilage destruction seen in peripheral joints.

The normal joint synovial tissue is dominated by ED2-positive resident macrophages. Polyethylene implants induced a much stronger foreign-body reaction than titanium implants as measured by the numbers of interfacial ED1-positive macrophages, especially in the arthritic joint. In the presence of a polyethylene implant the ED2-positive macrophages which are normally present in the synovial lining almost completely disappeared. One explanation may be that the resident ED2-positive macrophages have been exchanged with elicited ED1-positive macrophages. Another is that the macrophages have changed their phenotype; this has been suggested to occur depending on environmental circumstances and the state of activity. It is also known that if required ED2-positive resident macrophages can renew themselves by mitotic division. In contrast to polyethylene implants, in the presence of titanium implants the numbers of ED2-positive cells were retained with only small numbers of ED1-positive cells, indicating a much smaller disturbance of the synovial tissue. The implants had the same size and shape and similar surface topography making it unlikely that the observed differences were due to their physical form.

Our main finding was that the tissue response to titanium and polyethylene was vastly different in arthritic synovial tissue compared with normal tissue. This makes it more likely that the two materials interact differently with inflammatory cells or inflammatory mediators. The arthritic synovium is known to produce reactive oxygen intermediates (free radicals) and titanium to have unique interactions with such intermediates. Recent experiments in vitro have shown that titanium is oxidised by such reactive oxygen intermediates and transformed to less harmful titanium peroxo compounds which eventually degrade into titanium dioxide and hydrogen peroxide. It has been suggested that as a result of these mechanisms titanium acquires anti-inflammatory and, at the surface, antimicrobial properties by the formation of bactericidal titanium peroxo compounds.

Our results for subcutaneous and interstitial implants indicate that polyethylene as a biomaterial induces more inflammation than titanium as measured by the numbers of inflammatory cells at the interface. The effects of simultaneous adjuvant arthritis are not clear which may be due to the fact that the inflammation is more pronounced in the synovial tissue than other tissues.

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References


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