Polyethylene particles stimulate monocyte chemotactic and activating factor production in synovial mononuclear cells in vivo

An immunohistochemical study in rabbits

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We report that polyethylene particles can activate mononuclear cells within the joint to produce the monocyte chemotactic and activating factor (MCAF) and to a lesser degree interleukin 8 (IL-8) as judged by immunohistological staining. Polyethylene particles suspended in hyaluronic acid were injected weekly for 12 weeks into the right knee joint of New Zealand white rabbits. The average size of the particles was 7 (3–12) μm in diameter. The left knee joint was injected with hyaluronic acid as the control. The animals were killed after 13 weeks.

On the control side, the synovial membrane was histologically normal, without signs of inflammation. In the knees that were injected with polyethylene particles, histological analysis showed a weak inflammatory response, consisting of mononuclear cells, multi-nucleated giant cells and polyethylene particles. In the vicinity of the particles, the presence of mononuclear cells that were highly positive for MCAF was noted, whereas IL-8 was present in endothelial cells and in the lining layer, but not in cells in the vicinity of polyethylene particles, suggesting that polyethylene particles are able to activate cytokine metabolism in a differentiated way in the synovial monocytes.

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Polyethylene wear particles, released from endoprosthetic components during usage, induce a foreign body reaction in animals (Goodman et al. 1988, Howie and Vernon Roberts 1988). Furthermore, polyethylene particles (PEP) are connected with osteolysis, because loose arthroplastic components are surrounded by a pseudomembrane consisting of loose connective tissue and, in this layer, monocytic cells, giant cells and especially PEP are a frequent finding (Kwong et al. 1992, Schmalzried et al. 1993). This has led to the belief that PEP can initiate activation of macrophages, which results in loosening of the prosthetic component (Howie et al. 1988, Murray and Rushton 1990, Willert et al. 1990). This hypothesis has gained further support from a recent study indicating that the presence of T-cells is not necessary for the particles to induce inflammation (Goodman et al. 1994).

Cytokines of the chemokine family have been shown to play a central role in inflammatory responses, as this group of peptides is essential for creating a chemotactic gradient that attracts cells into areas of inflammation. The two most well known are probably interleukin 8 (IL-8) and monocyte chemotactic and activating factor (MCAF).

IL-8 belongs to the α-chemokine family and attracts T-cells and neutrophils (Larsen et al. 1989), whereas MCAF belongs to the β-chemokine family and attracts mainly monocytes (Yoshimura et al. 1989, Harigai et al. 1993).

Both IL-8 and MCAF can be produced by a great number of cells in the synovial membrane, including endothelial cells, monocytes and fibroblasts (Kristensen et al. 1991, Kristensen et al. 1993). Apart from attracting monocytes, IL-8 and MCAF also activate these cells and can thereby participate in the tissue destruction (Willems et al. 1989, Zachariae et al. 1990, Endo et al. 1991).

We investigated whether PEP activate the monocytes in vivo to produce IL-8 and/or MCAF. This was done by immunohistochemical detection of IL-8 and MCAF in the rabbit knee synovial membrane, after intraarticular injections with PEP.

Animals and methods

Experimental design

We used 6 New Zealand white rabbits, weighing between 3.0 and 3.5 kg. Weekly injections were given for 12 weeks in both knees under neurolept analgesia. To ensure intraarticular injection, a uniform, symmetrical swelling around the patella was noted and the patella could be tapped against the femur immediately after injection in all cases. 1 rabbit died of pneumonia 3 days after the first injection, leaving 5 rabbits for examination. None of the surviving rabbits showed signs of pain or limping, nor any clinical signs of infection. 1 week after the last injection, all animals were killed by an overdose of methohexital. Immediately postmortem, both knee joints were opened and samples for immunohistochemical examination were snap-frozen and stored at –80 °C. Finally, tissue was taken from all knees and cultured routinely for bacteria and fungus, but no signs of infection were observed.

Polyethylene particles

High molecular weight PEP were used after gas sterilization (formaldehyde) at 80 °C. In the right knee, 1 mL of viscous fluid was injected, containing 1 mg of PEP (approximately 7×10^9 particles) suspended in 3.5 mg hyaluronic acid per mL of phosphate buffered saline and with an average size of 7 (3–12) µm in diameter. The left knee was injected with 1 mL of the carrier substance as the control.

Tissue samples and immunohistochemical techniques

Samples for histological examination were fixed in 4% formaldehyde, microtomed to a thickness of 2 µm and stained with oil O red and hematoxylin-eosin. These specimens were used for assessment of inflammatory reaction and accumulation of PEP.

The IgG fraction of a rabbit anti-MCAF (Ra-α-MCAF) was biotinylated and both the Mo-α-IL-8 (clone WS-4) and Ra-α-MCAF were detected by the streptavidine-alkaline phosphatase technique. In short, 6 µm thick cryostat sections from each biopsy were cut and for MCAF staining they were fixed in 4% paraformaldehyde/96% ethanol v/v for 20 sec. This was followed by blocking for 20 min in 10% v/v of normal rabbit serum (NRS) in tris-buffered saline (TBS, 50 mM, pH 7.4). Sections were incubated with bio-Ra-α-MCAF (1.25 µg/mL), in 1% v/v NRS/TBS overnight at 4 °C. IL-8 detection was done after fixation in pure acetone for 8 min, blocking for 20 min in 10% v/v of normal goat serum (NGS) in TBS. Sections were incubated with WS-4 (12.5 µg/mL), in 1% v/v NGS/TBS overnight at 4 °C, followed by a biotinylated-goat anti-mouse 1:100 (Dako A/S, Denmark).

The biotinylated antibodies were detected by streptavidine-alkaline phosphatase (1:300, Amersham, UK). The staining was developed with fast red-TR salt (Sigma, St. Louis, U.S.A.) and levamisol (Sigma, St. Louis, U.S.A.) was added to inhibit endogenous alkaline phosphatases. After 5 min incubation, the reaction was stopped in TBS. Counterstaining was done in Mayer's hematoxylin. Positive staining is visualized by red coloration.

Specific staining was verified by: 1) substitution of the primary bio-Ra-α-MCAF or Mo-α-IL-8 antibody with biotinylated normal Ra IgG (1.25 µg/mL) or normal Mo IgG1 (12.5 µg/mL), 2) preincubation of the antibodies with 10 µg/mL of recombinant cytokine for 30 min at room temperature and 3) further dilution of the primary antibody, all resulting in negative staining. Presence of PEP was verified by examining the tissue in polarized light.

Data sampling and statistics

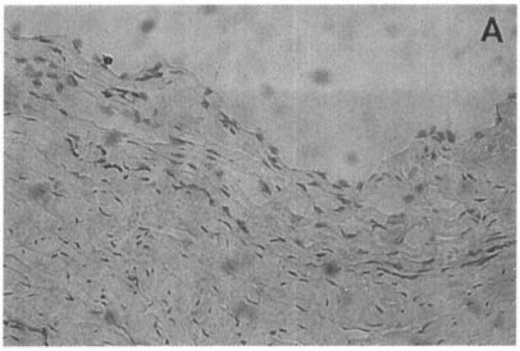
The number of positive cells was evaluated by light microscopy at a magnification of × 100, by counting 100 cells in each area of tissue examined. The synovial tissues were evaluated in 4 groups, according to the presence of IL-8/MCAF or PEP: 1) 0, if fewer than 5 cells were IL-8/MCAF positive or fewer than 5 PEP were present, 2) +, if 5-29 of the cells were positive or 5-29 PEP were present, 3) ++, if 30–60 of the cells were positive or 30-60 PEP were present and 4) +++, if more than 60 of the cells stained positive or if more than 60 PEP were present. The data were analyzed using Mann-Whitney's rank sum test.

Results

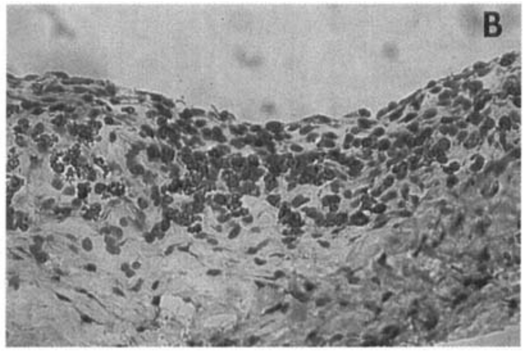
In the control joint, the synovial membrane was normal, without signs of inflammation. Single vessels were IL-8 positive, but no MCAF positive cells were observed (Figure 1). In the joints injected with PEP, the synovial membrane was characterized by a moderate inflammation consisting of mostly mononuclear cells, which were judged by morphology to be monocytes. Furthermore, a few multinucleated giant cells were observed, but only in 1 rabbit (no. 3) were lymphoid aggregates observed. The PEP were present beneath the lining layer and in the most superficial part of the interaggregated areas of the synovial membrane.

2 patterns of accumulated particles emerged: one with large areas of confluent particles under the synovial lining layer and another with a few PEP scat-

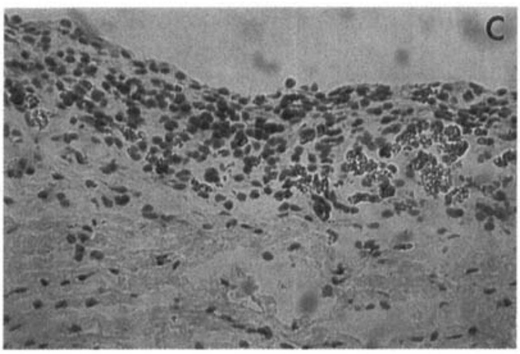
Figure 1. Distribution of MCAF in the rabbit synovial membrane after injection with polyethylene particles (PEP).



In the synovium from joints injected with vehicle only, the synovial membrane was normal, without signs of inflammation, and no MCAF positive cells were observed.

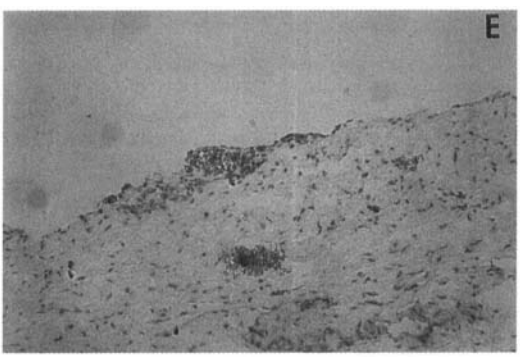


In the synovial membrane from joints injected with PEP, highly MCAF positive cells were found in the immediate vicinity of the PEP.



Normal mouse IgG failed to stain any cells (Alkaline phosphatase-anti-alkaline phosphatase technique, positive staining appears as red, hematoxylin counterstained; original magnification ×100).

Presence of PEP within the synovial membrane was verified by examining the tissue in polarized light (Same tissue as in B).



IL-8 was observed in vessels of both injected and control animals, but not in the vicinity of PEP.

Table 1. Polyethylene particles (PEP), IL-8 and MCAF positive cells in the rabbit synovium

Rabbit	Injected knee			Control knee		
	PEP	IL-8	MCAF	PEP	IL-8	MCAF
1	+++	0	++	0	0	0
2	+	0	+	0	0	0
3	+++	+	+++	0	0	0
4	+	0	+	0	0	0
5	++	0	+++	0	0	0

The amounts of PEP, IL-8 and MCAF positive cells were graded from 0 to +++ in the interaggregated part of the synovial membrane. The amounts of PEP and MCAF positive cells were increased in the PEP-injected knee (p 0.008).

tered throughout the membrane. In both cases, there was an increased number of cells that were positive for MCAF (p 0.008) (Table 1). These cells were observed in close connection or in the immediate vicinity of the particles, but were stained with the highest intensity around single or few PEP (Figure 1). The increase in MCAF positive cells was significant.

Apart from monocytes, vessels were also observed to be MCAF positive, although less intensely stained.

In the PEP-injected joints, IL-8 was mostly localized to the lining layer cells and vessels. In contrast to the MCAF staining, IL-8 was not present in the vicinity of PEP (Figures 1 and 2). There were differences in the number of IL-8 positive cells (Table 1).

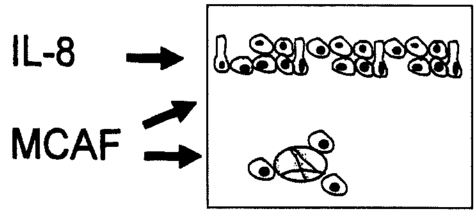


Figure 2. Staining pattern for IL-8 and MCAF in PEP-injected rabbits. In the PEP-injected animals, the synovial membrane IL-8 was present only in the lining layer and vessels, whereas highly MCAF positive cells were observed in the lining layer, vessels and in the vicinity of PEP.

Discussion

We found that PEP can induce the chemokine MCAF in vivo in the rabbit synovial membrane.

Since the presence of T-cells is not necessary for the PEP to create an inflammation, and as PEP are often seen in connection with giant-cell formation, the focus is on the monocyte (Goodman et al. 1994). Since MCAF can attract and activate monocytes, our studies link the PEP with a cytokine that can recruit monocytes. Furthermore, T-cells and neutrophils were not observed in the same areas as PEP, nor were the IL-8 positive cells. The presence of IL-8 positive cells in the synovial lining layer should probably be seen as a result of a general joint inflammation, but it is important to notice that IL-8 positive cells were not observed in the deeper parts of the synovial tissue. This would imply that PEP activate the mononuclear cells in a differentiated manner which favors a monocyte attraction that may be initiated by MCAF.

Monocytic cells have also been described in the pseudomembrane surrounding loose prosthetic components, often in close connection with PEP (Chiba et al. 1994). As MCAF can induce the secretion of the two osteolytic cytokines, IL-1α and IL-6 (Jiang et al. 1992), there is reason to believe that PEP in these areas can support the osteolytic process by triggering the cytokine cascade in monocytic cells.

Whether the induction of MCAF by PEP is a direct or indirect effect could not be determined by our study. PEP have also been reported to stimulate the secretion of IL-1 and TNF. Both of these cytokines are major inducers of MCAF (Jiranek et al. 1993, Deleuran 1994). However, injection of IL-1 or TNF results in a markedly inflamed synovium, often with cartilage and bone destruction, whereas MCAF injection creates a moderate inflammatory response, consisting mostly of monocytes (Akahoshi et al. 1993). The latter supports the view that PEP does not stimulate release of the proinflammatory cytokines like IL-1 or TNF in this situation, but further studies have to elucidate the precise mechanisms involved in the increased formation of MCAF in PEP-rich areas.

In retrieval studies, polyethylene wear particles have been estimated to have a diameter between 2 to 13 μm (Gelb et al. 1994), though this depends on the composition of the metallic part of the arthroplastic component (Gelb et al. 1994). Our particles had an average size of 7 μm in diameter and we observed only small-to-moderate inflammatory changes, localized to the uppermost layers of the synovial membrane. Other studies indicate that smaller particles, with a relatively greater surface, are more pro-inflammatory (Lee et al. 1992). Whether this also would increase the penetration and thereby the inflammatory response of PEP remains to be shown. Our study, however, shows that PEP with a size of 7 μm possess pro-inflammatory activities in vivo.

In conclusion, we found in an immunohistochemical evaluation of the synovial membrane that PEP can induce the monocyte chemotactic protein, MCAF, in vivo and we suggest that PEP, by induction of cytokines, take part in the inflammatory response.

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