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COMMUNICATIONS

Induction of collagenase and prostaglandin synthesis in synovial fibroblasts treated with monosodium urate crystals

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The inflammation of acute gout has been studied extensively and is mediated by interaction of crystals of monosodium urate monohydrate (MSUM) with phagocytic cells (Phelps & McCarty 1966; Weissmann & Rita 1972; McMillan et al 1980). In contrast, the pathophysiology of chronic gout has received much less attention and is poorly understood. Chronic gouty arthritis is characterized by deposition of tophi containing MSUM crystals in articular and periarticular tissues. Crystal deposition is associated with progressive erosion of bone, loss of cartilage and fibrosis which lead to joint destruction if left untreated (Sokoloff 1957). In the present study, we report that exposure of rabbit synovial fibroblasts to crystals of MSUM induces synthesis and release of high levels of collagenase and prostaglandin E_2, which are mediators of cartilage degradation and bone resorption respectively (Harris & Krane 1974; Robinson et al 1975). We suggest that the interaction of crystalline deposits of urate with resident synovial lining fibroblasts may contribute to joint destruction in chronic gouty arthritis.

Synovial tissue was dissected from the knee joints of 4–6 week old New Zealand white rabbits, then finely minced and dissociated enzymatically by sequential treatment with bacterial collagenase (4 mg ml^{-1}; 1 h; 37 °C) and trypsin (2·5 mg ml^{-1}; 3 h, 37 °C). After filtration through sterile gauze, cells were centrifuged, resuspended in Dulbecco's Modified Eagle's Medium (DMEM) containing 20% foetal calf serum (FCS), penicillin and streptomycin (50 μg ml^{-1}) and added to culture dishes. After two days, the dishes were washed and adherent fibroblast-like cells were subsequently grown to confluence in DMEM-10% FCS. Cells were cultured for up to 5 passages in DMEM-10% FCS.

Crystals of MSUM were prepared as described elsewhere (Hasselbacher 1979). Confluent monolayers of synovial fibroblasts were washed three times with Hanks Balanced Salt Solution (HBSS) and then incubated with MSUM for 72 h at 37 °C in serum-free DMEM containing 0·2% lactalbumin hydrolysate (LH). Collagenase activity was assayed by measuring lysis of reconstituted fibrils formed from ^{3}H-labelled guinea-pig skin collagen (Harris et al 1969). Specific cleavage products of collagen were identified using SDS polyacrylamide gel electrophoresis (SDS-PAGE) following viscometric assay of collagenase (McCroskery et al 1975). Radiolabelled metabolites of arachidonate were identified by thin layer chromatography (t.l.c.) and release of PGE_2 into culture medium was quantified by radioimmunoassay (McMillan, Fahey, Brinckerhoff, Hasselbacher, Harris, submitted for publication 1981).

Incubation of rabbit synovial fibroblasts with MSUM resulted in firm adherence of crystals to the cell surface. Crystals could not be removed by extensive washing but little ingestion of MSUM was observed using phase microscopy. Exposure of synovial fibroblasts to MSUM

Table 1. Release of collagenase and PGE_2 from rabbit synovial fibroblasts treated with MSUM. Approximately 6×10^5 cells, in 60 mm petri dishes, were incubated at 37 °C with 3 ml DMEM-0·2% LH containing a range of crystal concentrations. After three days, the culture medium was removed and the levels of collagenase and immunoreactive PGE_2 (i-PGE_2) were determined. Values represent mean (with s.d.) (n = 3). TCA-precipitable protein in cells was measured by the method of Lowry et al (1951). Control cultures contained 312 s.d. 30 μg protein/dish with (n = 3) and this was not significantly altered by treatment with any of the doses of crystals. One unit of collagenase activity degrades 1 μg reconstituted collagen fibrils min^{-1} at 37 °C. Latent collagenase was activated using 1·7 mM aminophenyl mercuric acetate.

MSUM (μg ml^{-1})	Collagenase (units mg^{-1} cell protein)	i-PGE_2 (ng mg^{-1} cell protein)
0	0	24 (4)
50	0	16 (5)
150	2·97 (0·77)	274 (36)
200	6·42 (1·35)	828 (163)
300	12·57 (2·32)	3444 (918)
500	16·00 (1·25)	7761 (1719)

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for 3 days in serum-free culture medium induced production of latent collagenase which could be activated by treatment with trypsin or amino phenyl mercuric acetate. No stimulation was produced by doses at which the crystals dissolve in aqueous medium ($\leqslant 50\,\mu g\ ml^{-1}$) but higher doses of MSUM provoked concentration-dependent synthesis and release of collagenase. Production of collagenase increased from undetectable levels in untreated control cultures to 16 units/mg cell protein in cells treated with $500\,\mu g\ ml^{-1}$ MSUM (Table 1).

In other experiments collagenase activity was monitored viscometrically at 27 °C and, after stopping enzyme action with EDTA, the products of collagen degradation were examined using SDS-PAGE. The initial mixture of α,β and γ chains was converted by activated medium from MSUM-treated cells to α^A, β^A,γ^A and α^B fragments which represent the three quarters and one quarter collagen cleavage products specifically formed by mammalian collagenases.

Exposure of rabbit synovial fibroblasts to MSUM also stimulated metabolism of arachidonic acid. In preliminary experiments cells were prelabelled with [^{14}C]-arachidonic acid before exposure to MSUM. T.l.c. scans of released lipids showed that MSUM induced the appearance of a major peak of radioactivity corresponding to authentic PGE_2 and a smaller peak, which migrated with PGB_2 and was probably formed by reduction of PGE_2 in the culture medium. The levels of PGE_2 released were determined by radioimmunoassay. Control cells released approximately 20 ng immunoreactive $PGE_2\ mg^{-1}$ cell protein during 3 days in culture but exposure to MSUM crystals stimulated immunoreactive PGE_2 levels by up to 300 fold to over $7\,\mu g\ mg^{-1}$ cell protein (Table 1).

Treatment of cells with crystals for three days at 37 °C did not significantly alter cell protein content but high doses of MSUM caused suppression of protein synthesis as measured by incorporation of radiolabelled leucine into cell protein. In a representative experiment cells treated with $500\,\mu g\ ml^{-1}$ MSUM incorporated 989 ± 63 counts $min^{-1}/100\,\mu g$ cell protein compared with a value for control cells of 2267 ± 204. MSUM also stimulated release of a cytoplasmic enzyme, lactate dehydrogenase, which suggests that crystal treatment resulted in some cytotoxicity. Since collagenase and prostaglandins are not stored intracellularly, the responses reported here are not merely a result of cell lysis. However, production of collagenase and PGE_2 in this system may be induced by a number of membrane-active agents (McMillan et al 1981) and it is possible that release of these mediators represents a reaction of synovial fibroblasts to harmful or injurious stimuli.

The levels of collagenase and PGE_2 released by rabbit synovial fibroblasts treated with MSUM are comparable to the high levels spontaneously produced by cultures of rheumatoid synovial cells (Dayer et al 1976). In rheumatoid arthritis, release of these mediators by synovial cells is believed to contribute to joint destruction and a variety of factors are likely to stimulate their synthesis. These include giant cell formation, phagocytosis of debris and a factor released by mononuclear cells (Brinckerhoff & Harris 1978; Werb & Reynolds 1974; Dayer et al 1977). If this experimental model using rabbit cells is relevant to the in vivo situation in man, we may infer that, in chronic gout, crystalline MSUM serves as a direct non-immunological stimulus to production of these mediators by resident synovial cells. We do not yet know whether the responses described here are specific for urate crystals or if other types of crystals of biomedical importance will induce similar responses. It will be important, in future studies, to determine whether release of collagenase and PGE_2 following fibroblast-crystal interaction may play a role in other crystal-induced disorders such as chondrocalcinosis, apatite deposition disease, silicosis, or asbestosis.

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