The cartilage-resorbing protein catabolin is made by synovial fibroblasts and its production is increased by phorbol myristate acetate

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Pig synovial fibroblasts were shown to produce a protein that caused live cartilage to resorb its proteoglycan matrix *in vitro*. Fibroblasts were obtained either from synovial tissue digests or by allowing them to grow out of explants. The population derived from the digests was homogeneous and free of macrophage-like cells after two passages, but was still producing the cartilage-resorbing protein after seven passages. The active protein was found to have M_r 20000 on gel filtration, and pI 4.8 on isoelectric focusing in polyacrylamide gel. It was indistinguishable from a protein with the same activity from pig mononuclear leucocytes, which has been called catabolin. Production of the protein was increased if the synovial fibroblasts were cultured with the tumour promoter phorbol 12-myristate 13-acetate. Fibroblasts from other sources (joint capsule and peritoneum) also apparently made the protein. The possibility that catabolin is the same as interleukin-1 is discussed: if they are, then the results suggest that fibroblasts can make an interleukin-1-like protein.

The resorption of connective tissues is a vital and continuing part of the normal processes of tissue growth and repair, and occurs in tumour invasion and inflammation. In rheumatoid arthritis, for example, chronic inflammation leads to extensive loss of articular cartilage. Cartilage in organ culture provides a simple means of studying connective-tissue resorption, since explants of cartilage are induced to degrade their matrix when cultured with synovial tissue (Fell & Jubb, 1977). The substance responsible for this effect has been called 'catabolin' (Dingle *et al.*, 1979) and is a small (M_r 20000) acidic (pI 4.6–5) protein (Saklatvala & Dingle, 1980; Saklatvala, 1981). An apparently identical protein is made by lectin-stimulated pig mononuclear leucocytes (Saklatvala & Sarsfield, 1982). Catabolin is defined and assayed by its ability to cause loss of matrix glycosaminoglycan from living cartilage *in vitro*. A human protein corresponding to it has been characterized from cultures of synovium and lectin-stimulated leucocytes (Saklatvala *et al.*, 1983).

It is possible that catabolin is the same as, or

closely related to, the monokine (mononuclear cell factor, MCF) that stimulates connective-tissue cells to make collagenase and PGE₂ (Dayer *et al.*, 1977, 1979), which is now thought to be identical with the lymphocyte-activating factor interleukin-1 (Mizel *et al.*, 1981; Postlethwaite *et al.*, 1983). The relationship between these proteins will be established when pure preparations are available: at present the names define biological activities.

In order to see which cells in the synovium could be making catabolin, we have studied pig synovial fibroblasts in culture. We report in the present paper that they can produce the protein and that their output of it is stimulated by PMA.

Materials and methods

Materials

DMEM, FCS, 0.05% trypsin/0.02% EDTA solution and all culture vessels were from Gibco. Clostridiopeptidase A, concanavalin A, PMA, papain, whale chondroitin sulphate (grade III), protein standards for chromatography, Fast Blue RR salt, maleate [mono-Tris (Trizma) salt], α -naphthyl acetate and PGE₂ were from Sigma. Dimethyl Methylene Blue was from Serva Feinbiochemica; Ultrogel AcA 54 and Ampholines were from LKB; Diaflo ultrafiltration apparatus and

Abbreviations used: DMEM, Dulbecco's modification of Eagle's medium; FCS, foetal-calf serum; PBS, phosphate-buffered saline (1 mM-NaH₂PO₄/18 mM-Na₂HPO₄/145 mM-NaCl, pH 7.4); PMA, phorbol 12-myristate 13-acetate; PGE₂, prostaglandin E₂.

PM 10 membranes were from Amicon; Ficoll 400 was from Pharmacia; and Hypaque sodium was from Sterling Research. Rabbit agglutinating antibody against sheep erythrocytes was from Sera Lab, and Carmine and Toluidine Blue were from George T. Gurr Ltd. Rabbit anti-PGE₂-bovine serum albumin was from Steranti Research, and [5,6,8,11,12,14,15(n)-³H]-PGE₂ came from Amersham.

All other chemicals were analytical-reagent or the best available grade.

Cell preparation and culture

Minced synovial tissue (intima plus some sub-intimal connective tissue) from the third and fourth metacarpo-phalangeal joints of freshly killed young pigs was used as a source of synovial cells. The minced tissue (approx. 2 mm pieces) was used to initiate cell cultures by the explantation method fully described by Vuorio (1977). The explants were cultured in DMEM supplemented with glutamine (60 µg/ml), penicillin (100 i.u./ml), streptomycin (100 µg/ml) and 20% (v/v) heat-inactivated FCS at 37°C in CO₂/air (1:19). The outgrowing cells reached confluence in about 20–30 days and were subcultured as follows: after the monolayers were washed in PBS, the cells were disaggregated by incubation at 37°C in a solution of 0.05% trypsin/0.02% EDTA in PBS for 5–10 min. The cells were then suspended in medium containing 10% FCS and plated at 0.3×10^5 cells/cm² and 10^5 cells/ml of medium. After the first passage, conditioned medium was collected and assayed for catabolin activity.

Further samples of synovial mince were used to prepare synovial cells by a method based on that of Dayer *et al.* (1976). Briefly, tissue was incubated in a solution of 4 mg of clostridiopeptidase A/ml in DMEM for 4 h at 37°C, and subsequently an equal volume of 0.05% trypsin/0.02% EDTA in PBS was added for a further 30 min. The resulting cell suspension was filtered (pore size 100 µm) and plated at 10^5 cells/ml in culture medium with glutamine and antibiotics as described above, plus 10% FCS, in plastic flasks. Confluence was reached in approx. 1 week, and the cells were cultured as described above. Medium was collected after serial passage and assayed for catabolin activity.

Explants of peritoneum and joint capsule of a newborn pig were used to initiate cell cultures as described. Confluence was reached in 3–4 weeks. Medium was collected from cells in passages 1–3 and assayed for catabolin activity.

PMA was added to groups of cultures of synovial fibroblasts in passage 7, and of peritoneal fibroblasts in passage 5. The medium was changed every 4 days and assayed for catabolin activity, for trypsin-activatable collagenase and for PGE₂. In some experiments, PMA (10 ng/ml) was added to conditioned medium from the synovial fibroblasts at the

end of the culture period, before assay of the medium for catabolin activity.

Assessment of the proportion of macrophages present in the cell cultures

Synovial cells derived by digestion were examined as primary cultures and at subsequent passages. The cells were plated at low density ($<10^4$ cells/ml), allowed to recover from the effects of trypsin treatment for 24–48 h and then subjected to the following tests for macrophage function.

(a) *Phagocytosis*. Monolayers were exposed to a suspension of Carmine in culture medium + 10% FCS for 2 h at 37°C. The cultures were washed three times in PBS and cells removed from the plate with trypsin as described. Cells containing ingested Carmine were counted with a haemocytometer.

(b) *Non-specific esterase*. The α -naphthyl acetate method (based on that of Gomori, 1952) for demonstrating the presence of esterases was used. A mixture of 25 mg of Fast Blue RR salt in 50 ml of Trizma maleate (200 mM) buffer, adjusted to pH 7.6, and 20 mg of α -naphthyl acetate in 2 ml of methoxy-ethanol was added to living cells. The cells were incubated in the dark at 37°C for 15 min, rinsed in tap water and examined.

(c) *Fc receptors*. The presence of receptors for the Fc region of immunoglobulin G was assayed by a method based on that of Gordon & Cohn (1970). Sheep erythrocytes, sensitized by incubation for 30 min with rabbit antiserum to sheep erythrocytes, were added at a concentration of 5×10^8 cells/ml to living synovial cells. After incubation at 37°C for 30 min the cultures were thoroughly washed and the cells examined for the presence of erythrocyte rosettes.

Biological assay of cartilage degradation

Discs of bovine nasal cartilage (4 mm diameter $\times$ 1 mm thick) were cultured for 6 days in culture medium with 5% FCS. Test samples were included in the medium throughout. At the end of the culture the discs were digested with papain, and the glycosaminoglycan in the media and discs was assayed spectrophotometrically by using dimethyl Methylene Blue (Humbel & Etringer, 1974; Farndale *et al.*, 1982). The assay was calibrated with chondroitin sulphate. Catabolin activity is expressed as the percentage of the total cartilage glycosaminoglycan that is released into the medium. For some assays cortisol was included in the cartilage cultures. This procedure has been fully described elsewhere (Saklatvala, 1981).

Fractionation of used synovial-cell medium

The medium was concentrated by ultrafiltration in a Diaflo apparatus with a PM 10 membrane and chromatographed on Ultrogel AcA 54 under conditions described in the appropriate Figure legends.

Fractions active in the cartilage assay were focused in 7.5%-polyacrylamide gel cylinders (5.9 mm × 60 mm) containing Ampholine gradients and assayed for activity. This procedure has been fully described (Saklatvala & Sarsfield, 1982).

Other methods

Collagenase was measured by the method of Cawston & Barrett (1979), by using [^{14}C]acetylated rat skin collagen. Samples were treated with trypsin (200 $\mu\text{g}/\text{ml}$) for 30 min at 37°C, followed by soya-bean trypsin inhibitor (1 mg/ml). A unit is defined as the amount of enzyme degrading 1 μg of collagen/min at 37°C. PGE_2 was assayed by specific radioimmunoassay (Jaffe & Behrman, 1979).

Protein was determined by the method of Oyama & Eagle (1956). Cell numbers were determined by use of a haemocytometer.

Results

Morphology of cell cultures

When cells were prepared by explanting tissue, the initial outgrowth consisted of a mixed population of

cells, including small (20 μm diam.) cells with the appearance of macrophages, and larger spindle-shaped fibroblastic cells. The small macrophage-like cells gradually disappeared from the cultures, so that when confluence was reached (at approx. 20–28 days) all the cells resembled fibroblasts.

Similarly, adherent synovial cells prepared by enzymic digestion were heterogeneous in primary culture. At least two types of cells were present; small (20 μm diam.) round cells with a prominent nucleus, and larger fibroblastic cells containing a large nucleus, with two or three prominent nucleoli. Confluence was reached in 6–8 days. After the first passage, the cell population appeared homogeneous, consisting of the larger fibroblastic cells, which assumed a 'swirling' pattern at high density (Fig. 1).

Macrophage markers on synovial cells

Because of the need to study a homogeneous cell population, it was important to assess the proportion of contaminating macrophages in the cultures. Table 1 shows the distribution of cells bearing macrophage markers. Cells bearing macrophage markers

Fig. 1. *Pig synovial cells grown from explants of synovium*

The monolayer was fixed in 4% (w/v) formaldehyde solution at pH 5 and stained in 0.1% Toluidine Blue in 0.1 M-sodium borate, pH 9. Cells in primary cultures at day 40 appear homogeneous. Magnification $\times 250$.

Table 1. *Macrophage markers in serially passaged synovial cells from tissue digests*

The mean percentage of the total number of cells demonstrating formation of Fc rosettes after incubation with sensitized sheep erythrocytes, or showing a positive reaction for non-specific esterase, or active in phagocytosis of Carmine particles, is shown $\pm$ S.E.M. Cells were given 24 h to recover from the effects of trypsin treatment. Numbers in parentheses indicate the numbers of separate assessments made.

Passage no. ...	0		1	2	3	7
	Day 1	Day 6				
Fc receptors	5.6 $\pm$ 2 (5)	3.5 $\pm$ 0.9 (5)	5 $\pm$ 0.6 (5)	2.8 $\pm$ 0.7 (8)	1.6 $\pm$ 0.5 (6)	0.075 $\pm$ 0.075 (4)
Non-specific esterase	12 $\pm$ 2 (5)	2.7 $\pm$ 0.4 (4)	4 $\pm$ 1.2 (5)	0.5 $\pm$ 0.1 (3)	1.1 (1)	0.5 $\pm$ 0.2 (3)
Phagocytosis	5.7 $\pm$ 0.6 (4)	2.3 $\pm$ 1 (4)	2.8 $\pm$ 0.8 (7)	0.3 $\pm$ 0.2 (4)	0 (1)	0.1 $\pm$ 0.1 (4)

comprise, at most, 5% of the population after the first passage, and are absent after the second.

Production of cartilage-resorbing activity by synovial fibroblasts

Supernatants from synovial cells after serial passage induced release of glycosaminoglycan from living cartilage. Cartilage killed by freezing and thawing three times was not induced to resorb, demonstrating that the effect was chondrocyte-dependent. Cultures of synovial cells derived by digestion were examined up to 7 passages, by which time the cells had been in culture for approx. 10 weeks (Fig. 2). Explant-derived cells were equally effective in producing cartilage-degrading activity after passage. There was no relationship between the growth rate of the cells and their output of active material, its production depending more on cell number. The continuing production of active material after serial passage of the cells strongly suggests that it arose from fibroblasts. Pig macrophages in culture replicate only very slowly indeed, and their numbers become progressively depleted (Owen *et al.*, 1975). If such cells were responsible for the production of the active substance, they would need to make ever-increasing quantities of it per cell in order to account for the amounts found after serial passage.

Production of cartilage-resorbing activity by other fibroblasts

Fibroblasts derived from explants of the peritoneum and joint capsule of a newborn pig also produced cartilage-degrading activity for at least 5 weeks after initiation of the cultures, through successive passages (Table 2).

Characterization of the active component from synovial fibroblasts

Medium from explant-derived cells from passages 2–5 was concentrated by ultrafiltration and chromatographed on Ultrogel AcA 54. The elution

Fig. 2. *Production of cartilage-resorbing activity by synovial cells from tissue digests after serial passage* Synovial cells derived by enzyme digestion were maintained through successive passages in DMEM + 10% FCS. The medium was changed every 4 days and assayed at a concentration of 500 μ l/ml of fresh culture medium for cartilage-resorbing activity on bovine nasal cartilage. Each set of blocks shows the mean amount of glycosaminoglycan release (vertical bars are S.E.M.) induced in five cartilage discs by medium from cells of each passage, as compared with that produced by control medium (shaded blocks). Medium conditioned by synovial cells induced significantly more release than did control medium; on the basis of the Student's *t* distribution, *P* values were less than 0.001 for unmarked blocks, and less than 0.01 for blocks marked with asterisks.

Table 2. *Glycosaminoglycan release from bovine nasal cartilage induced by culture medium from fibroblasts from joint capsule and peritoneum*

The Table shows the percentage release $\pm$ S.E.M. of glycosaminoglycan from five discs of bovine nasal cartilage cultured with 50% (v/v) medium conditioned by cultured fibroblasts derived from explants of peritoneum or joint capsule of newborn pig. The first passage took place at 3–4 weeks, and subsequent passages were performed every 7 days. The cells were plated at each passage at 10^5 cells/ml of medium. Glycosaminoglycan release induced by conditioned medium is significantly greater than that produced by control medium. *P* values were calculated on the basis of the Student's *t* distribution.

	Culture period (days)	Glycosaminoglycan release from cartilage (%)	<i>P</i>
Control medium		20 $\pm$ 7	
Peritoneal fibroblast medium			
1st passage	3	54 $\pm$ 7	<0.001
2nd passage	3	31 $\pm$ 5	0.05–0.1
3rd passage	3	78 $\pm$ 4	<0.001
On dead cartilage		15 $\pm$ 0.7	
Joint-capsule fibroblast medium			
1st passage	7	46 $\pm$ 3	<0.001
2nd passage	3	30 $\pm$ 1	0.001–0.01
On dead cartilage		14 $\pm$ 3	

volume of the active component corresponded to M_r 20000 (Fig. 3a) and was the same as that of the leucocyte catabolin.

The active fractions were subjected to isoelectric focusing in a wide-range gradient of pH 3–10, and focused at about pH 4.8. Narrow-range isoelectric focusing (pH 4–6) revealed an active component that focused at pH 4.5 (Fig. 4). For comparison, similar active material prepared from lectin-stimulated leucocyte supernatants was run in a parallel gel. The active components of each preparation focused with an identical profile. On the basis of molecular size and charge, the active component from the fibroblasts was indistinguishable from leucocyte catabolin.

Effect of PMA on catabolin production by fibroblasts

Addition of PMA (3–30 ng/ml) to cultures of digest-derived synovial or explant-derived peritoneal fibroblasts caused marked stimulation of their output of cartilage-resorbing activity (Fig. 5). Dead cartilage was unaffected. The medium conditioned by the synovial cells was serially diluted for assay on cartilage, so that the maximum dose of PMA carried over into the bioassay was 15 ng/ml. At this concentration PMA did not cause significant glycosaminoglycan release from the cartilage, but at concentrations of 30 ng/ml or higher it did. There was no increase in cell number or total cell protein relative to the control values during exposure of the fibroblasts to PMA. Some signs of toxicity were apparent at the highest dose, including withdrawal of cell processes and the appearance of basophilic inclusions in the cytoplasm of some cells.

It was possible that the apparent stimulation of the fibroblasts was simply due to the same amounts of cartilage-resorbing protein being more active on the cartilage in the presence of the PMA that was carried over into the assay. To exclude this possibility, PMA was added to the medium of unstimulated fibroblasts at the end of the conditioning period, but this caused no increase in the activity of such medium. It was concluded that the PMA must be stimulating the fibroblasts to make more of the active material.

Since PMA is known to stimulate fibroblastic cells to produce collagenase and prostaglandins (Brinckerhoff *et al.*, 1979; Brinckerhoff & Harris, 1981), these substances were measured. Amounts of both were markedly increased (Table 3).

Synovial fibroblasts in third passage were stimulated with PMA, and the active component in the medium was characterized. On gel-filtration chromatography its elution volume corresponded to a M_r of 20000 (Fig. 3b) and its pI was 4.5–5 (Fig. 6a). It was not distinguishable from the leucocyte catabolin focused in a parallel gel (Fig. 6b). It was concluded that the unstimulated and stimulated fibroblasts made the same active protein.

Discussion

Catabolin was originally identified as a product of synovial connective tissue in organ culture which stimulated resorption of cartilage proteoglycan. The aim of this investigation was to find out whether cells derived from this connective tissue would produce the same protein, and particular attention was paid

Fig. 3. Gel filtration of conditioned medium of pig synovial fibroblasts

(a) Cells grown out of explants were passaged at approx. day 28 and plated in DMEM + 10% FCS in 75 cm flasks. Medium (1 litre) was pooled from cultures in passages 2–5, concentrated 100-fold and chromatographed on a column (100 cm $\times$ 4.4 cm) of Ultrogel AcA 54, which was eluted with PBS at 60 ml/h. Fractions (15 ml) were dialysed against DMEM and assayed at 400 μ l/ml of culture medium for activity on bovine nasal cartilage. Cortisol (0.1 μ g/ml) was included in the cartilage assay. (b) Digest-derived synovial fibroblasts in third passage were plated in 75 cm² plastic flasks at 3×10^5 cells/cm² and 10^3 cells/ml. On day 4, the medium was changed to include 10 ng of PMA/ml. Medium plus PMA was replenished every 4 days until day 16. Conditioned medium was pooled (120 ml), concentrated 40-fold by ultrafiltration and chromatographed on a column (90 cm $\times$ 1.5 cm) of Ultrogel AcA 54, eluted with PBS at 6 ml/h. Fractions (3 ml) were dialysed and assayed at 120 μ l of culture medium as in (a). Results are the means for quintuplicate assays $\pm$ S.E.M. The A_{280} (----) was also recorded. The arrows indicate the elution volumes of: a, Dextran Blue; b, bovine serum albumin (M_r 68 000); c, soya-bean trypsin inhibitor (M_r 21 500); d, cytochrome c (M_r 12 500); e, aprotinin (M_r 6500); 'x' marks the elution volume of the leucocyte catabolin, prepared as described by Saklatvala & Sarsfield (1982).

Fig. 4. Isoelectric focusing of active material from pig synovial fibroblasts and from mononuclear leucocytes

The active fractions (55–61 inclusive) from gel filtration (Fig. 3a) were pooled, concentrated 40-fold, dialysed against 1% glycine and focused in polyacrylamide gels in a pH 4–6 gradient. Crude mononuclear-leucocyte culture medium was chromatographed on Ultrogel AcA 54 (as in Fig. 3) and active fractions were pooled, concentrated and focused in a parallel gel. Gel slices were homogenized in 1.5 ml of PBS, dialysed against PBS overnight, and subsequently against two changes of DMEM. The samples were added to culture medium to a concentration of 250 μ l/ml and assayed on bovine nasal cartilage in the presence of cortisol (0.1 μ g/ml). Results are means of quintuplicate assays. ●, Leucocyte catabolin; ○, fibroblast catabolin; —, pH.

to studying a homogeneous cell population obtained by repeated passaging. The synovial fibroblasts derived from explants or by digestion continued after serial passage for many weeks to produce material active in the cartilage assay. The primary cultures of synovial cells from tissue digests contained some cells which we assume to be of the macrophage type, since they possessed characteristic markers. These cells disappeared from the cultures during the first two passages, so that by the time of the third passage the population was made up solely of cells of fibroblastic morphology. When cells were grown from explants of synovium, the first passage did not take place for approx. 28 days, by which time macrophage-type cells derived from both blood and tissue have disappeared from the cultures.

A possible cause of the proteoglycan release induced by fibroblast-conditioned medium was that proteinases from the fibroblasts were acting directly on the cartilage matrix. The results of the experiments in which the medium was incubated with dead cartilage showed no evidence of such a direct enzymic effect. This was not surprising, since the cartilage was cultured in the presence of serum, which contains proteinase inhibitors. Another pos-

Fig. 5. Stimulation by PMA of catabolin production from synovial fibroblasts

Digest-derived synovial fibroblasts at passage 7 or peritoneal fibroblasts in passage 5 were plated in DMEM plus 10% FCS at 0.3×10^5 cells/cm² and 10^5 cells/ml in 35 mm plastic culture dishes. After 4 days (day zero), the medium was changed to control medium (□) or to include PMA at 3 ng/ml (●), 10 ng/ml (○) or 30 ng/ml (■). The medium was changed every 4 days and assayed at three concentrations (12.5–50%) for catabolin activity. Results are shown for (a) synovial and (b) peritoneal fibroblasts, and are means $\pm$ S.E.M. for quintuplicate assays on pooled medium from four to eight cultures. The PMA alone at the dilutions tested had no effect on the release of glycosaminoglycan by the cartilage.

sibility was that the chondrocytes were activating a latent proteinase from the fibroblasts, but characterization of the active component showed that it

Table 3. *Production of collagenase and PGE₂ by pig fibroblasts stimulated with PMA*

The Table shows mean values $\pm$ S.E.M. measured in medium from cultured (a) pig synovial and (b) peritoneal fibroblasts maintained in the presence of PMA. The procedure is fully described in the legend to Fig. 5. Numbers in parentheses indicate the numbers of separate cultures assessed. Abbreviation: ND, not determined.

	PMA (ng/ml)	Collagenase (units/ml)		PGE ₂ (ng/ml)	
		Days 0-4	Days 4-8	Days 0-4	Days 4-8
(a) Synovial fibroblasts	0	10.6 $\pm$ 3.9 (4)	ND	155 $\pm$ 39 (4)	ND
	3	44.4 $\pm$ 1.4 (4)	ND	447 $\pm$ 29 (4)	ND
	10	47.6 $\pm$ 10.4 (4)	ND	438 $\pm$ 28 (4)	ND
	30	67.2 $\pm$ 6.4 (4)	ND	629 $\pm$ 39 (4)	ND
(b) Peritoneal fibroblasts	0	0 (4)	0 (5)	14.6 $\pm$ 0.2 (4)	6 $\pm$ 0.3 (5)
	3	21.2 $\pm$ 2.9 (4)	27.3 $\pm$ 2.4 (5)	1219 $\pm$ 113 (4)	1374 $\pm$ 179 (5)
	10	25.2 $\pm$ 1.8 (4)	36.5 $\pm$ 3.4 (5)	1381 $\pm$ 99 (4)	2180 $\pm$ 50 (5)
	30	20.3 $\pm$ 0.5 (4)	36.3 $\pm$ 3 (5)	1219 $\pm$ 69 (4)	2770 $\pm$ 249 (5)

Fig. 6. *Isoelectric focusing of active material from conditioned medium from synovial fibroblasts stimulated with PMA*

Active fractions (22-28 inclusive) from gel-filtration chromatography (Fig. 3b) were pooled, concentrated 12-fold by ultrafiltration, and subjected to isoelectric focusing in polyacrylamide gels as described in Fig. 4. A preparation of leucocyte catabolin was focused in a parallel gel (as in Fig. 5). Gels were sliced and eluted as described, and the eluates (250 μ l/ml of medium) were assayed for catabolin activity. Cortisol (0.1 μ g/ml) was included in the assay. Results are means of quintuplicate assays $\pm$ S.E.M.. (a) Fibroblast catabolin (O); (b) leucocyte catabolin (●); ---, pH.

was a protein of about 20000 *M_r* that was closely similar to the leucocyte catabolin. There was no evidence of an active component corresponding to the proteoglycan-degrading neutral proteinase produced by synovium (Murphy *et al.*, 1981; Cambray *et al.*, 1981). For these reasons we conclude that the synovial fibroblasts produced catabolin. Since the protein is also made by mononuclear leucocytes (Saklatvala & Sarsfield, 1982), it does not seem to be a product unique to a particular cell type.

PMA stimulated the fibroblasts to make more of the protein, as well as more collagenase and PGE₂. PMA is known to stimulate synovial fibroblasts to produce these last two substances (Brinckerhoff *et al.*, 1979; Brinckerhoff & Nagel, 1981; Brinckerhoff & Harris, 1981). The increased cartilage-resorbing activity observed in the culture supernatants of fibroblasts stimulated with PMA could possibly have been explained by increased production of fibroblast proteinases, but characterization of the active component made by the PMA-stimulated fibroblasts again revealed only a protein corresponding to catabolin. PMA is a tumour promoter *in vivo*, and induces a variety of effects in cultured cells, reminiscent of viral transformation (Pollack *et al.*, 1974; Wigler & Weinstein, 1976; Driedger & Blumberg, 1977). It is an interesting possibility that cell transformation could be associated with enhanced production of catabolin. We have only characterized the protein from synovial fibroblasts, but the experiments performed with peritoneal and joint-capsule fibroblasts suggest that they also make it.

In the introduction it was mentioned that catabolin and interleukin-1 could be the same or closely related proteins. If this were so, then the results presented here suggest that fibroblasts can make an interleukin-1-like protein. Although interleukin-1 is generally considered to be a product of macrophages, there have been reports of interleukin-1-like material (assayed by its ability to enhance proliferation of mouse thymocytes) arising from PMA-stimulated 3T3 fibroblasts (Okai *et al.*, 1982) and

the keratinocyte cell line Pam 212 (Luger *et al.*, 1981).

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