Partial purification of a factor from human synovium that possesses interleukin 1, chondrocyte stimulating and catabolin-like activities

Peter R. Elford, Judith E. Meats, R. Michael Sharrard and R. Graham G. Russell*

Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, Beach Hill Road, Sheffield S10 2RX, England

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Human synovial explants in culture release material that stimulates the production of prostaglandin E_2 (PGE_2) and several extracellular enzymes by human chondrocytes. Fractionation of conditioned medium by gel filtration revealed a protein of approx. 15 kDa, which in addition to stimulating production of PGE_2 and plasminogen activator by human articular chondrocytes, possessed interleukin 1 activity and induced cartilage degradation. Further purification using iso-electric focussing again showed co-elution of these activities with a major p*I* of 6.9 and a minor p*I* of 5.1–5.3. This study indicated that human synovium releases a facor that is closely related to or identical with interleukin 1 and suggests that this protein may participate in cellular interactions that occur within the rheumatoid joint.

Cartilage *Chondrocyte* *Interleukin 1* *Prostaglandin E* *Rheumatoid arthritis* *Synovium*

1. INTRODUCTION

Although the causes of rheumatoid arthritis remain obscure, there is now considerable evidence that some of the pathological events may be mediated by interaction between cell types within the rheumatoid joint. Authors in [1] showed that when porcine synovium and cartilage were maintained in the same organ co-culture system, destruction of cartilage matrix took place. They suggested that cultured synovial tissue released a factor (later termed 'catabolin') [2] which induced chrondrocytes to degrade their own matrix, since degradation only occurred if the cartilage was viable. Further work by authors in [3] showed that conditioned medium from human synovium also stimulated cartilage-resorption whilst studies in our laboratory [4] indicated that such medium stimulated the production of PGE_2 and PA by isolated human chondrocytes, an activity we attributed to 'synovial factor' (SF). It was found [5] that normal blood monocytes release in culture a soluble factor (termed mononuclear factor, MCF) that stimulated the production of prostaglandin E and collagenase by cells isolated from human rheumatoid synovium. Factors derived from synovium and mononuclear cells may be important mediators of rheumatoid arthritis since they are produced by, and act upon, cell types known to be present at potential sites of inflammation and connective tissue degradation.

Investigation into the identity of MCF revealed a protein that appeared to be very similar to IL-1 [6], a monokine previously shown to modulate a variety of immunological functions [7,8]. Subsequent studies in our laboratory [9,10] using partially purified human IL-1 have shown that IL-1 possesses the ability to stimulate the production of

* To whom correspondence should be addressed

Abbreviations: PGE_2, prostaglandin E_2; IL-1, interleukin 1; PA, plasminogen activator; FCS, fetal calf serum; LAF, lymphocyte activating factor

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enzymes and PGE_2 by isolated human articular chondrocytes and to induce both degradation of cartilage and bone resorption, activities that had previously been attributed to MCF [11–13]. Since there appeared to be many similarities between the connective-tissue activators, we have investigated further the profile of biological activity of a more purified SF, which unlike the other factors, had not been resolved further than gel filtration. Purification from the conditioned medium of normal human synovial explants was carried out using gel filtration followed by iso-electric focussing, and biological activity assessed using the standard assays for IL-1 (murine thymocyte proliferation), catabolin (release of glycosaminoglycan from explants of bovine nasal cartilage) and MCF (production of PGE_2 and enzymes by isolated human chondrocytes).

2. EXPERIMENTAL

2.1. *Production and purification of SF*

Specimens of normal synovium were obtained from adult human knee joints after surgical amputation for lower limb ischaemia or trauma. The synovium was freed from surrounding tissues by microdissection and cultured as small explants (approx. 20–30 mg tissue), cultures contained 1 g (wet wt) of tissue per 10 ml minimal essential medium supplemented with 10% FCS plus 100 U/ml penicillin and 100 μg/ml streptomycin. After 3 days of culture, conditioned medium containing SF was removed and stored at $-20°C$. Medium from 6–8 SF preparations was pooled, freeze-dried and reconstituted in 50 mM ammonium acetate buffer (pH 7.4) and applied to an 80×4.4 cm column of BioGel P30, equilibrated in 50 mM ammonium acetate. Fractions eluting with an apparent molecular mass of 15 ($\pm$ 2.5) kDa were pooled and dialysed against 0.5% (w/v) glycine. The dialysed material was then further fractionated in an LKB preparative iso-electric focussing apparatus using a beaded gel containing 1.6% (w/v) ampholines (pH 3.5–10) and 0.4% (w/v) ampholines (pH 7–9). After completion of focussing the gel bed was divided into 30 fractions and the material contained in each fraction eluted with 1.5 ml distilled water, the pH recorded, and the gel further eluted with 1.5 ml phosphate-buffered saline. Fractions were dialysed against phosphate-buffered saline (3 changes) and stored at $-20°C$ until assay.

2.2. *Human chondrocyte culture techniques*

Slices of normal articular cartilage were taken from the femoral and tibial condyles of human knee joints obtained after surgical amputation for lower limb ischaemia or trauma. Chondrocytes were dispersed by sequential digestion and cultured as in [9]. Prostaglandins in the culture media were measured by radioimmunoassay [14] utilising an antiserum with specificity towards PGE_2 (Steranti Research, St Albans, England). Production of plasminogen activator by chondrocytes was measured as in [15].

2.3. *Cartilage resorption assay*

This was performed as described [9], results being expressed as % total chondroitin sulphate released from fragments of bovine nasal cartilage over an 8-day incubation period.

2.4. *IL-1 assay*

IL-1 activity was measured by the enhancement of lectin-stimulated mouse thymocyte proliferation (the lymphocyte activating factor, LAF, assay). Murine thymocytes (2×10^6 per well) were cultured for 72 h in 200 μl RPMI 1640, supplemented with 5% FCS, antibiotics and 2.5×10^{-5} M mercaptoethanol in microtest trays. Cells were stimulated with test materials and phytohaemagglutinin (1 μg/ml). Cultures were pulse-labelled for the last 18 h with 0.5 μCi/well [^{3}H]thymidine and harvested with an automatic cell harvester ('Titertek', Flow Laboratories, Irvine, Scotland).

3. RESULTS

Fig.1 shows gel filtration profiles of SF conditioned medium. The activity originally ascribed to the factor, namely the ability to stimulate production of PGE_2 and PA by chondrocytes, co-elutes with IL-1 (LAF) activity at approx. 15 kDa, which is the characteristic molecular mass of IL-1. The 15-kDa peak also induces the release of chondroitin sulphate from bovine nasal cartilage. A smaller activity at higher molecular mass is present, which has also been observed after gel filtration of human synovial catabolin, and may be caused by aggregation of the protein of the main

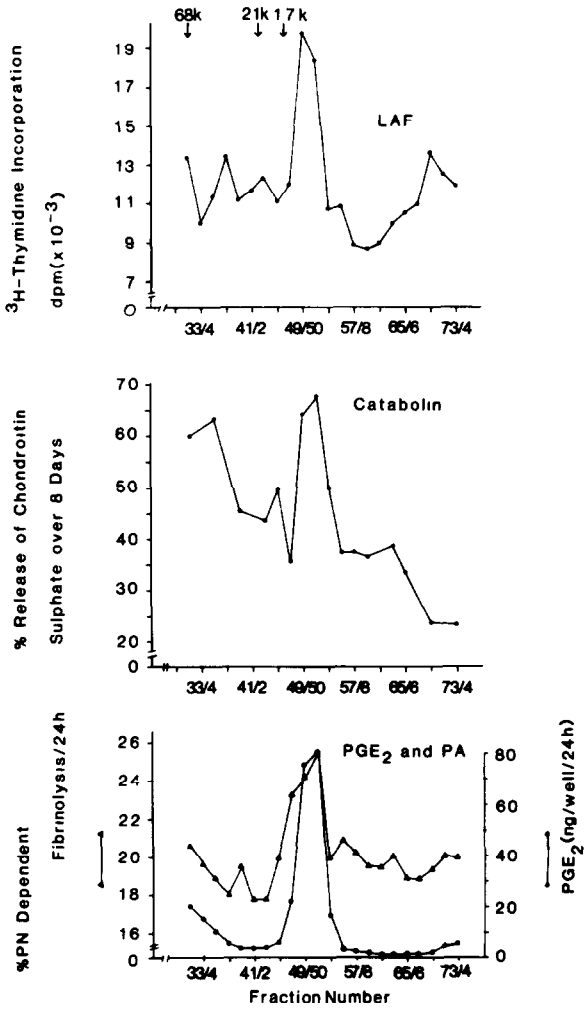

Fig.1. Gel filtration chromatography of IL-1 (LAF), cartilage-degrading and chondrocyte-stimulating activities. 20 ml of the synovial explant culture fluid concentrate was chromatographed over BioGel P30 in 50 mM ammonium acetate, pH 7.4. Fractions were tested for IL-1 activity at 2% dilution, chondrocyte-stimulating activity at 4%, and for the ability to induce cartilage degradation at 10% dilution.

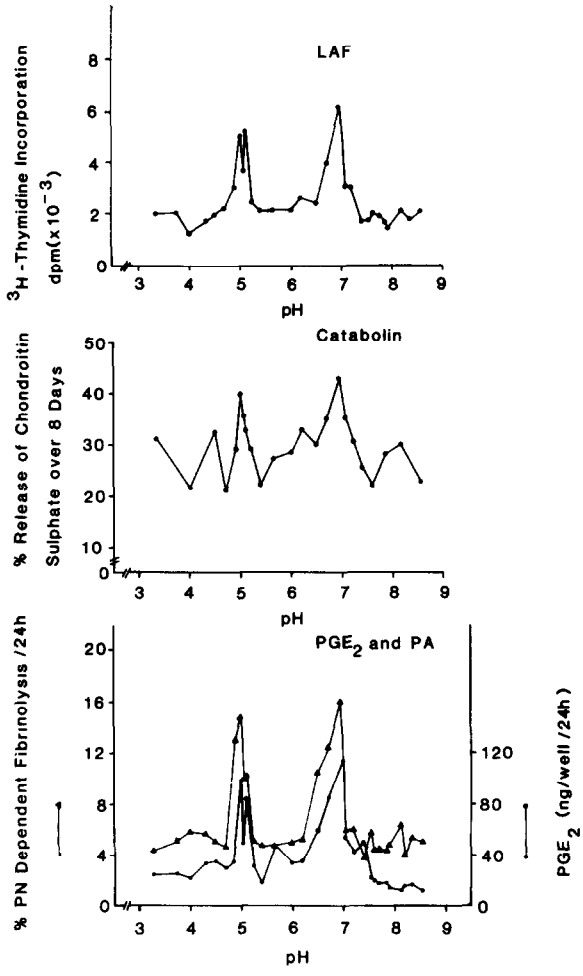

Fig.2. Distribution of the biological activities of SF on iso-electric focussing. The 15 (± 2.5) kDa SF-rich fraction obtained by gel filtration chromatography of synovial explant conditioned medium was further fractionated by iso-electric focussing and the fractions dialysed against phosphate buffered saline. Fractions were tested for IL-1 activity at a dilution of 2%, for chondrocyte-stimulating activity at 4% dilution and for the induction of cartilage degradation at 10% dilution.

peak [3]. Fig.2 shows the results from further fractionation of SF by isoelectric focussing of the 15-kDa peak obtained by gel filtration. A major peak of IL-1 activity occurs at a p*I* of 6.9 and a minor peak is present between p*I* 5.1 and 5.3. This pattern of iso-electric points is typical of that observed for human IL-1 [10]. Both the cartilage-degrading and chondrocyte-stimulating activities follow the IL-1 profile. This suggests that the same molecule, or closely related family of molecules, is responsible for all the activities observed.

4. DISCUSSION

By gel filtration and iso-electric focussing, SF exhibited properties that did not distinguish it from IL-1. To our knowledge, this is the first report of the production of IL-1 activity by human

synovial tissue. The presence of IL-1 activity in the synovial fluids has been observed [16–18]. In [17] no correlation was found between the number of macrophages, and the amount of IL-1 present in synovial fluids. It is therefore possible that the synovium is a significant source of the IL-1 present in the joint fluids. IL-1 derived from the synovium may be produced by the type A synovial lining cells, which are thought to belong to the mononuclear phagocyte system and possess receptors for complement factor C3 and IgG Fc [19–21], or from cells not of the monocyte/macrophage lineage, as has been described for other tissues [7].

Our finding suggest that the synovium may contribute to the pathology of rheumatoid arthritis not only by the release of proteolytic enzymes but also through the secretion of IL-1. These results, together with those in [9], mentioned above, show that cartilage degradation can be mediated by IL-1 through the induction of chondrocyte catabolic activity. This could occur in vivo following cellular activation by IL-1 derived from the invading synovial pannus. Such IL-1 would also affect immune cells present either in the synovial fluids or in the synovium itself. Cells resembling T-lymphocytes and plasma cells containing IgG, as well as monocytes, have been identified in rheumatoid synovial tissue [22–24]. Co-operation between these cell types may contribute to the characteristic chronicity of the disease through the continued activation of monocyte/macrophages and subsequent release of further IL-1 into the joint tissues.

Another interesting finding is that the major cartilage-degrading activity of SF occurs at a different p*I* to that previously described for catabolin. Partially purified human synovial catabolin is reported to be an acidic protein, with a p*I* of 5.2–5.3, there being no apparent biologically active species with neutral p*I* [3]. SF however, has a major peak with a p*I* of 6.9 in addition to the acidic peak characteristic of catabolin. The reason for this discrepancy is at present unknown, although reports on catabolin have only involved the use of the cartilage-degradation assay, and further investigation of its biological activity by the assays for IL-1 and MCF used above, would help to clarify its relationship to IL-1/MCF/SF.

Our finding that human synovium produces a factor biochemically indistinguishable from IL-1 suggests that cellular interactions within the rheumatoid joint, leading to the destruction of cartilage, may be mediated by the family of related peptides comprising IL-1. Confirmation of this hypothesis however, awaits further characterisation of IL-1 by monoclonal antibodies and by peptide and gene sequencing.

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