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Altered patterns of protein phosphorylation in articular chondrocytes treated with interleukin-1 or synovial cytokines

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Cultures of lapine articular chondrocytes were exposed to purified, human, recombinant interleukin-1α or partially purified preparations of lapine, synovial, cytokines in the presence of [^{32}P]orthophosphate. After 30 min incubation, phosphoproteins were extracted from the cells, separated by two-dimensional gel electrophoresis and visualized autoradiographically. Analysis of the autoradiograms revealed that interleukin-1 and the synovial factors produced marked changes in the pattern of protein phosphorylation. The synovial cytokines induced many of the same changes as interleukin-1, as well as a number of unique changes. This finding is consistent with the notion that, in addition to interleukin-1, synoviocytes secrete other cytokines which modulate the metabolism of chondrocytes. These data support the idea that signal transduction in chondrocytes responding to interleukin-1 involves the activation of one or more protein kinases.

Interleukin-1; Synovial cytokine; Chondrocyte-activating factor; Protein phosphorylation; Signal transduction; Protein kinase

1. INTRODUCTION

Articular chondrocytes undergo a process of activation following treatment with interleukin-1 (IL-1). Cultures of activated chondrocytes synthesize and secrete large amounts of prostaglandin E_2 (PGE_2) and various neutral proteinases [1–4], while their synthesis of matrix constituents is concomitantly suppressed [5,6]. Such changes may underlie the ability of IL-1 to induce the autolytic digestion of living cartilage both in vivo [7] and in vitro [1]. These processes are likely to occur during the development of arthritis, leading to erosion of the articular surfaces.

Synovium is a major intraarticular source of IL-1. Although macrophages [8] and polymorphonuclear leukocytes [9–11] contribute to the synovial production of IL-1 during inflammatory episodes, the resident synovial fibroblasts also secrete appreciable amounts of this cytokine [4,12,13]. Moreover, we have proposed that, in addition to IL-1, synoviocytes secrete other cytokines which modulate the activation state of chondrocytes [14]. For convenience, these unfractionated mixtures of synovial cytokines are referred to as chondrocyte-activating factors (CAF) [4,12].

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Abbreviations: IL-1, interleukin-1; PGE_2, prostaglandin E_2; CAF, chondrocyte-activating factor

Although the physiological responses to IL-1 have been widely studied, little is known of the signal transduction mechanisms involved. IL-1α and IL-1β share a common cell surface receptor [15,16], but no second messenger has yet been clearly identified. In preliminary experiments it has not proved possible to link cAMP or Ca^{2+} to the induction of neutral metalloproteinases in chondrocytes [12]. However, IL-1 increases intracellular concentrations of cAMP in lymphocytes and fibroblasts [17,18], generates inositol triphosphate in macrophages [19] and stimulates the production of diacylglycerol in Jurkat cells [20]. It remains possible that certain cellular responses to IL-1 are mediated through novel signalling mechanisms.

As all known signal transduction mechanisms at some stage activate a protein kinase, we thought it pertinent at this stage to determine whether IL-1 and CAF alter the pattern of protein phosphorylation in chondrocytes. Only two other published articles report the changes in protein phosphorylation in response to IL-1. Both of these involve white cells [21,22].

2. EXPERIMENTAL

2.1. *Reagents*

The following reagents were purchased from the indicated suppliers: interleukin-1α (Genzyme, Boston, MA); tissue culture media, sera, antibiotics, etc. (Gibco, Grand Island, NY); tissue culture plasticware (Fisher Scientific, Pittsburgh, PA); young adult New Zealand white rabbits (Green Meadows Rabbitry, Murrysville, PA); [^{32}P]orthophosphate (New England Nuclear, Boston, MA); electrophoretic equipment and supplies (BioRad, Richmond, CA).

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2.2. *Methods*

Monolayer cultures of articular chondrocytes were prepared from the knee and shoulder joints of New Zealand white rabbits, as described by Green [23]. Cells were seeded into 24-well plates or 35 mm Petri dishes, at a concentration of 10^5 cells per cm^2, and used when confluent, without subculture. Synovial CAF was prepared from cultures of the HIG-82 synovial cell line [24] as described elsewhere [12].

2.3. *Protein phosphorylation*

Growth medium was removed and, after washing the cell sheet twice with Gey's solution, replaced by phosphate-free minimal essential medium without serum. Cells were metabolically labelled with 200 μCi/ml ^{32}P-orthophosphate for 3.5 h, after which time IL-1α (20 U/ml) or CAF (25 μl/ml; 200 μg protein) were added to certain cultures. All cultures were returned to the incubator for a further 30 min. Media were then removed, the cell sheet washed twice with ice-cold Gey's solution and processed for two-dimensional gel electrophoresis, according to the method of O'Farrell [25], using a p*I* range from pH 3–10 in the first dimension, and a 5–15% polyacrylamide gradient in the second.

These studies revealed multiple effects of IL-1 and CAF on the patterns of phosphoproteins (data not shown). As these changes were too complex for resolution by eye, triplicate samples were sent to Protein Databases Inc. (Huntington Station, New York) for analysis. Autoradiograms were scanned with an Eikonix 78/99 camera system, and the images processed with the PDQUEST computer analysis based upon the initial design and algorithms of Garrels et al. [26].

3. RESULTS AND DISCUSSION

Representative autoradiograms are shown in fig.1. Two-dimensional gels prepared from unactivated, control chondrocytes contained 845 different ^{32}P-labelled spots that could be distinguished by computer analysis

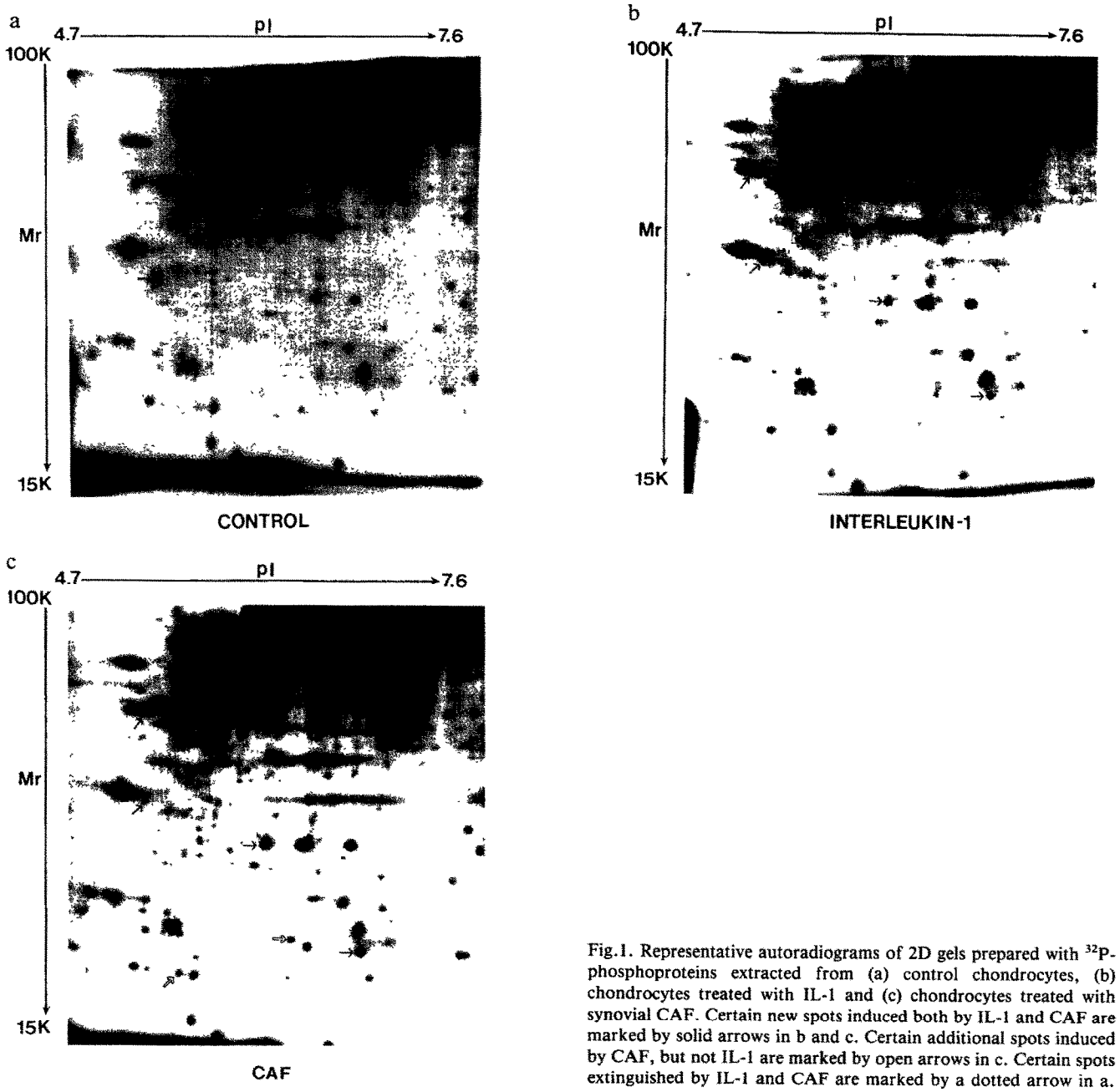
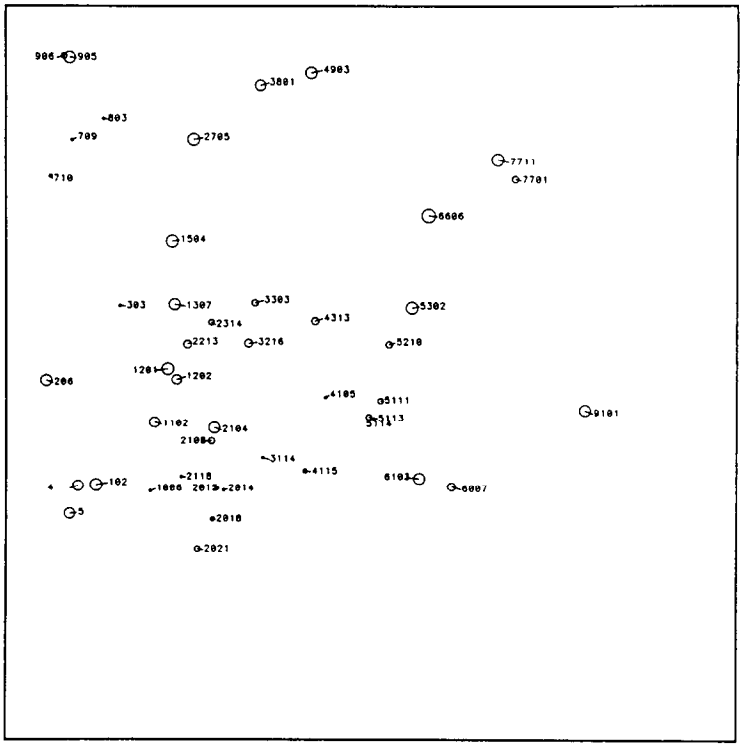


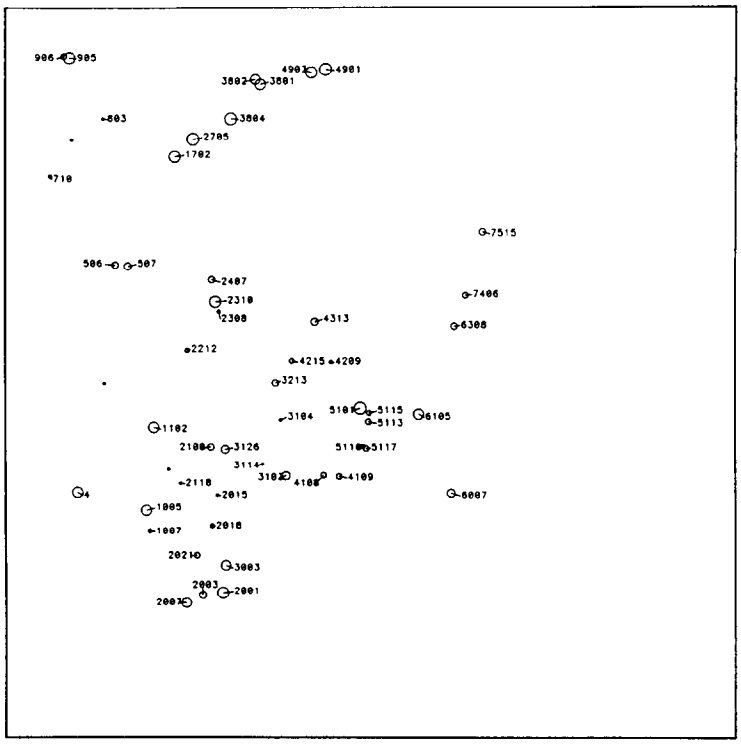
Fig.1. Representative autoradiograms of 2D gels prepared with ^{32}P-phosphoproteins extracted from (a) control chondrocytes, (b) chondrocytes treated with IL-1 and (c) chondrocytes treated with synovial CAF. Certain new spots induced both by IL-1 and CAF are marked by solid arrows in b and c. Certain additional spots induced by CAF, but not IL-1 are marked by open arrows in c. Certain spots extinguished by IL-1 and CAF are marked by a dotted arrow in a.

a E43 CONTROL VS IL-1 mpdi01624



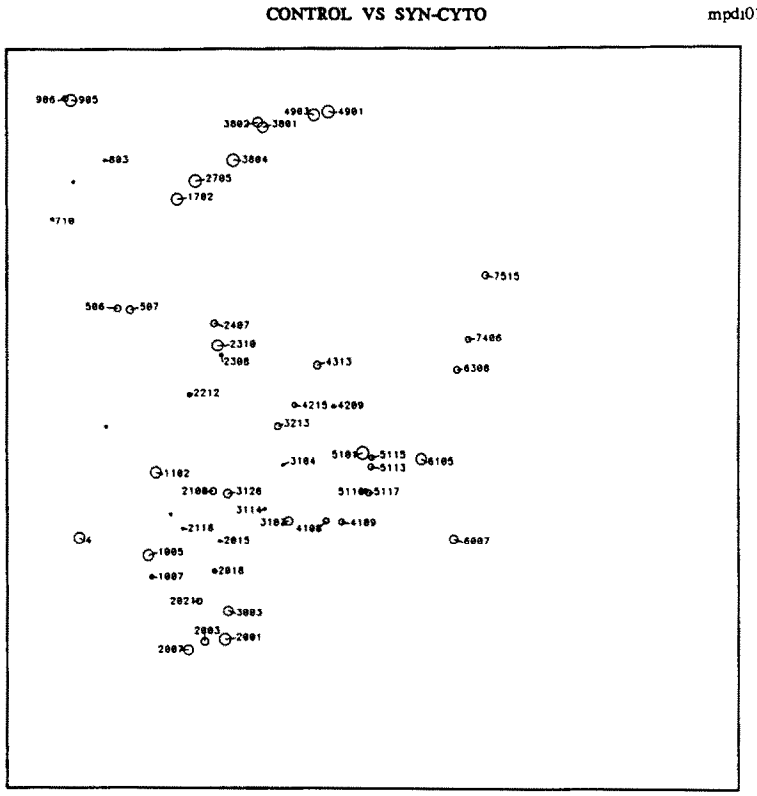
Number of Spots Shown on This Map: 45

b E43 CONTROL VS SYN-CYTO mpdi01624



Number of Spots Shown on This Map: 53

Fig.2. Differences between autoradiograms are shown on these computer-generated charts. The size of each circle is indicative of the magnitude of the difference. (a) Control vs IL-1; (b) control vs CAF; (c) IL-1 vs CAF.



of the autoradiograms. Addition of IL-1 or CAF induced a number of new phosphoproteins, while certain phosphoproteins were observed only in material extracted from control cells (fig.1).

Comparison of triplicate autoradiograms prepared from control chondrocytes and chondrocytes treated with IL-1, revealed 45 statistically significant ($P < 0.05$) differences (fig.2a). Comparison of autoradiograms prepared from control chondrocytes and chondrocytes treated with CAF revealed 53 differences (fig.2b). As indicated in fig.1b and c, many of these differences were common to cells treated with IL-1 and CAF (fig.2c). However, there were 28 differences between the phosphoprotein patterns produced by IL-1 and CAF. These data are consistent with the notion that synovial CAF consists of IL-1 together with additional cytokines which produce their own alterations in cellular phosphoproteins.

The differences produced by IL-1 and CAF presumably reflect changes in the activities of one or more protein kinases or phosphatases in chondrocytes. The magnitude and rapidity of the changes render unlikely more complicated explanations based, for example, upon the induction of new proteins or altered substrate availability. Future investigations should be directed towards identifying the kinases or phosphatases responsible for these changes and determining their role in signal transduction. In preliminary experiments, we have not found inhibitors of protein kinase C to inhibit the ability of IL-1 to increase the synthesis of PGE_2 and neutral metalloproteinases by chondrocytes [27].

NOTE ADDED IN PROOF

Kaur and Saklatvala (FEBS Lett. (1988) 241, 6–10), studying protein phosphorylation in human dermal fibroblasts exposed to IL-1α and TNF-α, reported similar results to ours. In particular, there is a striking resemblance between their 'triad of 27 kDa phosphoproteins (p*I* 6.0, 5.7 and 5.5)' and the similar, if not identical, triad appearing on our 2-D gels. The most acidic of these three proteins, each of which is phosphorylated in response to IL-1 and CAF, is arrowed in our figs 1b and c.

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