Effect of interleukin-1β on the production of cathepsin B by rabbit articular chondrocytes

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Rabbit articular chondrocytes in monolayer culture were stimulated with human recombinant interleukin-1β. Under the influence of the cytokine the intracellular pool of the cysteine endopeptidase cathepsin B was increased by a 2–4-fold factor, while enzyme secretion was not stimulated at a significant level. Under the same conditions, the secretion of collagenase, measured as an internal control, was stimulated about 6-fold. The effects of interleukin-1β were compared to those caused by phenotypic modulation. Chondrocytes modulated by serial subcultures in monolayer secreted more cathepsin B, but less collagenase than differentiated cells (cultured within collagen gels). Thus, interleukin-1β and phenotypic modulation affected differently two endopeptidases which are relevant in the pathogenesis of osteoarthritis.

Cathepsin B; Collagenase; Articular chondrocyte; Interleukin-1β; Cell culture; Rabbit

1. INTRODUCTION

The activation of articular chondrocytes by cytokines of various origin has received great attention in recent years because of the possible implication of this phenomenon in the pathogenesis of rheumatic diseases. The degradation of cartilage components has been largely attributed to chrondrocytic collagenase and other metal-dependent endopeptidases stimulated by cytokines from synovium [1], monocyte-macrophage lineage cells [2], polymorphonuclear leukocytes [3] or of autocrine origin [4]. Cartilage degradation has been classically observed using organ cultures of this connective tissue [5] and the factor responsible for chondrocyte activation has originally been described as 'catabolin' [6]. The co-identity of catabolin and mononuclear cell factors with IL-1 is now established [7]. After this discovery, the effects of purified IL-1 upon chondrocyte metabolism were considered in more detail [7–21].

Another way in which chondrocytes are stimulated to synthesize and secrete proteins not normally produced by differentiated cells, is phenotypic modulation. The interest for proteins from phenotypically-modulated chrondrocytes has mainly focused on collagen types [22], while only recently attention has been paid in this context to cartilage-degrading endopeptidases [23–26].

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Abbreviations: IL-1, interleukin-1; DMEM, Dulbecco's modified Eagle's medium

This study shows the effect of IL-1 on the production of cathepsin B by articular chondrocytes and compares it with the known collagenase-stimulating activity of the cytokine, as well as with the effects caused by phenotypic modulation of the cells.

2. MATERIALS AND METHODS

Recombinant human IL-1β was a gift of Dr K. Vosbeck, Ciba-Geigy Ltd, Basel Switzerland; indomethacin, dexamethasone and cycloheximide were from Sigma, St. Louis, MO, USA, and diclofenac sodium was from Ciba-Geigy Ltd, Basel, Switzerland. The source of all other materials was specified previously [23,24].

Articular chondrocytes were prepared from the pooled hip, knee and shoulder cartilages of New Zealand rabbits and cultured in monolayers or in collagen gel suspensions [23]. Primary cells were cultured to confluence (4–9 days), divided in portions and stored frozen until further use. After thawing, the cells were transferred into 25 cm^2 culture flasks with 1×10^6 cells/flask in 5.0 ml DMEM supplemented with 10% fetal calf serum. Confluent cells were trypsinized and transferred to 24-well culture plates (16 mm diameter) with 1.0×10^5 cells/well in 1.0 ml DMEM/10% fetal calf serum. For collagen gel cultures, the pellets were prepared by polymerizing 1.0 ml of collagen solution, containing 1.0×10^5 cells, that were incubated with 2.0 ml DMEM/10% fetal calf serum. When IL-1 was added to the cells immediately or within 2–3 days after this transfer, no effects upon collagenase stimulation could be observed. We could establish that this lack of response was due to destruction of IL-1 receptors on the chondrocyte cell surface after trypsinization, and that a period of 5–6 days was necessary to completely restore the receptors. Therefore, any experiment with added substances was initiated 6 days after transferring the cells into 24-well plates or to collagen gels. Test substances were added after dilution of stock solutions with serum-free DMEM at the following final concentrations: IL-1β, 10 ng/ml (0.59 nM); diclofenac sodium, 1.27 ng/ml (4 nM); indomethacin, 0.36 μg/ml (1 μM); dexamethasone, 0.39 μg/ml (μM). All tests were set up in quintuplicate in serum-free DMEM (time zero). The culture medium was withdrawn after 24 h and 48 h and replaced with fresh

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serum-free medium containing the appropriate substances. Enzyme assays were performed in each of the five media/test collected at 24 h intervals giving 3 time groups that will be designated as the 24, 48 and 72 h media. This method was chosen to allow detection of possible time-dependent effects upon cell stimulation. Each of the bars shown in the figures represents the cumulative enzyme activities of 5 distinct cultures measured at 3 time intervals for a total of 15 measurements/bar.

Cathepsin B and collagenase assays in supernatants and cell homogenates were performed as described [23,24,27,28]. The collagenase assays were performed in the presence of *p*-aminophenylmercuric acetate to measure the total enzyme, i.e. latent plus active, and were set up within 2 h after sampling to avoid activity losses upon freezing and thawing. The media were then stored at −20°C for subsequent cathepsin B assays since no losses of activity could be observed upon storage for several months and subsequent thawing.

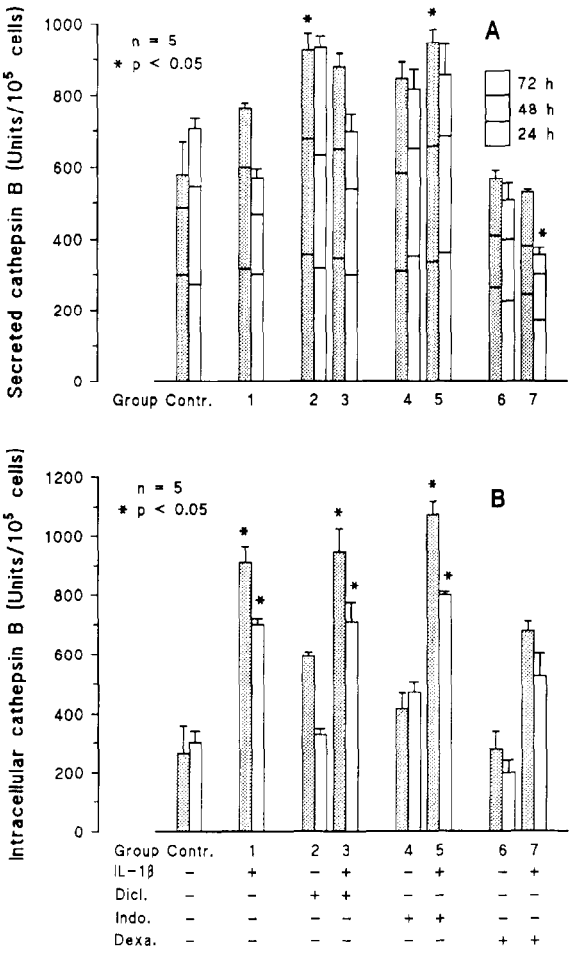

Fig. 1. Effect of IL-1β, diclofenac (Dicl.), indomethacin (Indo.) and dexamethasone (Dexa.) on cathepsin B production by rabbit articular chondrocytes in monolayer culture. The contributions to the cumulative secreted activity of the 24, 48 and 72 h media are shown in panel A. The intracellular enzyme was measured after withdrawing the last medium at 72 h. The void bars and the shaded bars represent two independent series of experiments. Statistical analysis was performed with the distribution-free Kruskal–Wallis test for assessing significant differences between groups, followed by the multiple comparison Dunn's test of several treatment groups to a control, at a significance level $\alpha = 0.05$ (two-tailed). As appropriate of non-parametric analysis, data are shown as median with standard error of the median. The asterix indicates a significant difference from the control.

Our collagenase assay with reconstituted collagen fibrils [27,28] measured EDTA-inhibitable enzyme in excess over possibly present collagenase inhibitors [25,26], which were not investigated.

3. RESULTS AND DISCUSSION

In this study, aimed at showing variation of enzyme activities in either direction, chondrocytes from a first subculture were always used to set up the experiments. This was necessary, since freshly prepared chondrocytes secreted barely measurable cathepsin B levels [23]. The cells, whose properties are shown in Figs 1–3, could be induced to either secrete more or less cathepsin B, depending on the treatment, and were thus chosen as a flexible investigation tool. IL-1β as well as diclofenac, indomethacin and dexamethasone did not stimulate further cathepsin B secretion by articular chondrocytes (Fig. 1A). The purpose of adding these substances was that of testing the effects of two inhibitors of prostaglandin biosynthesis (diclofenac and indomethacin) and the effect of a glucocorticoid (dexamethasone). The relatively small, although statistically significant variations with respect to the control noted in one experiment were not confirmed in another, independent experiment (Fig. 1A). On the contrary, the accumulation of cathepsin B in intracellular granules was regularly stimulated by a factor 2–4 in the presence of IL-1β, an effect not contrasted by prostaglandin inhibitors (Fig. 1B). For checking the validity of the experimental conditions, the known effects of IL-1β and of the other test substances upon collagenase secretion were measured. As shown in Fig. 2, collagenase secretion was enhanced by a factor of $\simeq 6$ in the presence of IL-1β, an effect not counteracted by diclofenac and in-

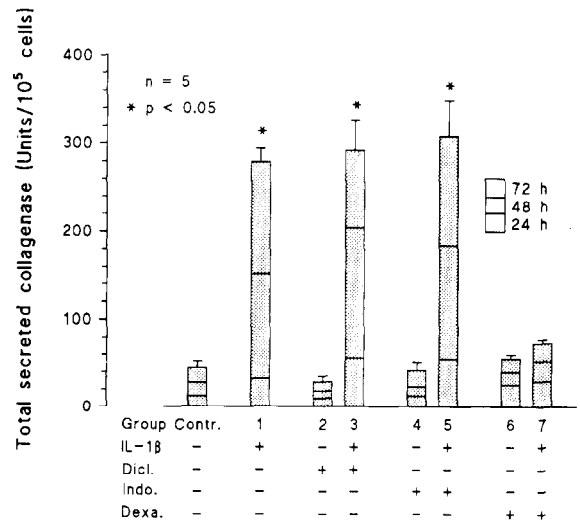

Fig. 2. Effect of IL-1β, diclofenac (Dicl.), indomethacin (Indo.) and dexamethasone (Dexa.) on collagenase secretion by rabbit articular chondrocytes in monolayer culture. The experiment was the same as that with shaded bars in Fig. 1. Other details and statistics as in Fig. 1.

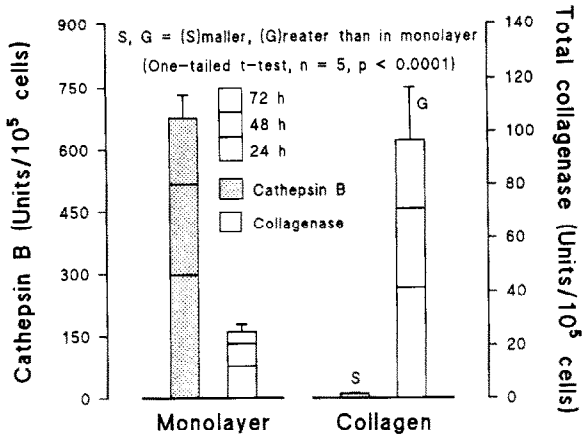

Fig. 3. Comparison of cathepsin B and collagenase secretion in monolayer and collagen gel cultures. The same cell stock was cultured with both methods and the media were collected at 24 h intervals for 3 days. Since statistical analysis was performed with a parametric method, bars represent the cumulative activity as mean with SD.

domethacin at concentrations known to inhibit prostaglandin synthesis. This confirms that prostaglandins and metallo-endopeptidases are independently regulated in chondrocytes [19,29], and the same concept seems to be valid for the intracellular accumulation of cathepsin B. Intracellular collagenase was never found. Since the collagenase data reproduce those published by other investigators [8,9,11,14,19], the conditions used for cathepsin B measurements in Fig. 1 are likely to be safe. Enzyme secretion was directly dependent upon protein synthesis, since cycloheximide (1 μg/ml) strongly reduced both collagenase and cathepsin B levels (data not shown, for cathepsin B see [24]). Dexamethasone could efficiently counteract the stimulatory effect of IL-1β upon collagenase secretion (Fig. 2) and could also moderately, but significantly interfere with the intracellular accumulation of cathepsin B (Fig. 1B). These effects are in line with literature data [30,31] that suggest a mechanism of action of dexamethasone at the transcriptional level by decreasing the mRNAs of various proteins through receptor-mediated inhibition of gene expression.

The same cell stock used in the experiments of Figs. 1 and 2 was used to show in parallel the effect of

Table I

Effects of IL-1β stimulation and subculture modulation on cathepsin B and collagenase production by rabbit articular chondrocytes. (↓, ↑, ↑↑, ↑↑↑) qualitative scale for reduction or stimulation, from moderate to very strong

	IL-1β	Modulation	
Cathepsin B	Intracellular	↑	↑↑↑
	Secreted	Unchanged	↑↑↑
Collagenase	(Secreted)	↑↑	↓

phenotypic modulation upon the secretion of cathepsin B and collagenase. Chondrocytes cultured within a three-dimensional matrix, such as a collagen gel, are considered to represent the differentiated phenotype while cells cultured in monolayer are representative of a modulated phenotype [22]. Fig. 3 compares parallel experiments on the secretion of cathepsin B and collagenase by chondrocytes cultured in monolayers and within collagen gels. With respect to the monolayer cultures, cathepsin B secretion was drastically reduced in the collagen gel cultures. Collagenase secretion was relatively low in monolayers, but significantly higher than blanks, and was nearly quadruplicated at a very significant statistical level in collagen cultures. When the cells cultured in monolayer (experiments in Fig. 3) where further passaged to a fifth subculture, cathepsin B secretion was triplicated, whereas collagenase secretion remained constant (not shown, data confirming previous results [23]).

IL-1 has been implicated in the pathogenesis of osteoarthritis [32] and has been localized in human osteoarthritic synovium as well as cartilage [33], thus suggesting a role of inflammation in the pathophysiology of osteoarthritis. However, other aspects of osteoarhritis, such as the switch from collagen type II to type I synthesis [22] and the production of cathepsin B [23,34], we believe can now be ascribed to phenotypic changes on the part of the chondrocytes rather than to IL-1 stimulation (see Table I). It is thus possible that these two routes of chondrocyte activation in osteoarthritis either coexist or that they represent episodes occurring independently at different times during the development of the disease.

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