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Interleukin-1 α modulates collagen gene expression in cultured synovial cells

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The effects of porcine interleukin-1 (IL-1) α on collagen production were studied in cultured human rheumatoid synovial cells. Addition of 0.05–5 ng of IL-1/ml into the cultures resulted in a dose-dependent decreased rate of collagen released into the medium over 24 h. To determine whether this inhibition was due to secondary action of prostaglandin E_2 (PGE_2) secreted in response to IL-1, cultures were incubated in presence of various inhibitors of arachidonate metabolism. Depending on the cell strains, these inhibitors were able to suppress or diminish the effect of IL-1, suggesting that PGE_2 is involved in the mechanism. Depression of collagen production caused by IL-1 mainly affected type I collagen and therefore led to a change in the type I/type III collagen ratio in the extracellular medium. Steady-state levels of mRNA for types I and III procollagens were estimated by dot-blot hybridization and compared with the amounts of respective collagens produced in the same cultures. IL-1 generally increased procollagen type I mRNA, but to a variable extent, as did indomethacin (Indo). Depending on the cell strain, the combination of indo and IL-1 could elevate the mRNA level of type I procollagen compared with Indo alone. These results did not correlate with the production rate of collagen in the medium, which was diminished by exposure to IL-1. The level of mRNA for collagen type III was not greatly changed by incubation with IL-1, and a better correlation was generally observed with the amount of type III collagen found in the medium. These data suggest that an additional control mechanism at translational or post-translational level must exist, counterbalancing the stimulatory effect of IL-1 on collagen mRNA transcription. It is likely that IL-1 could modulate the production of collagen in synovial cells by an interplay of different mechanisms, some of them limiting the effect of primary elevation of the steady-state mRNA level.

INTRODUCTION

Studies from several laboratories have established that cytokines produced by blood mononuclear cells or tissue macrophages can modulate the synthesis of collagen by connective-tissue cells (reviewed by Freundlich *et al.*, 1986). Interleukin-1 (IL-1) has been proposed as one of these regulatory factors (Krane *et al.*, 1985; Pujol *et al.*, 1985; Bhatnagar *et al.*, 1986; Goldring *et al.*, 1986; Kähäri *et al.*, 1987). This monokine is already known to stimulate both prostaglandin E_2 (PGE_2) and collagenase release in synovial cells (Mizel *et al.*, 1981), cartilage and bone resorption (Saklatvala *et al.*, 1984), secretion of neutral metalloproteinases by articular chondrocytes (Gowen *et al.*, 1984), synthesis of hyaluronic acid (Hamerman & Wood, 1984) and proliferation of fibroblasts (Schmidt *et al.*, 1982). However, there is still some controversy on the effects exerted by IL-1 on collagen synthesis. We initially showed that a purified IL-1 could depress the production of collagen by rabbit articular chondrocytes (Pujol *et al.*, 1984) or human synovial cells (Pujol *et al.*, 1985). More recently, we also observed a similar inhibitory effect in dermal fibroblasts (Bhatnagar *et al.*, 1986). This latter result was in agreement with data obtained by Whiteside *et al.* (1984). In contrast, others have found that IL-1 preparations enhance collagen synthesis by dermal fibroblasts (Kähäri *et al.*, 1987) and synovial cells (Krane *et al.*, 1985).

These discrepancies may be due to differences in experimental procedure, since factors such as the source of target cells, culture conditions, concentrations of IL-1 used, duration of the incubation and method of analysis may all affect the experimental outcome. Any variation in the conditions can lead to completely different results (Freundlich *et al.*, 1986). In this respect, an important point to consider is the fact that IL-1 stimulates PG in connective-tissue cells, so that secondary effects of PG could mask the underlying action of IL-1. PGE_2 inhibits collagen synthesis, possibly by increasing intracellular cyclic AMP (Baum *et al.*, 1978, 1980; Clark *et al.*, 1982). A stimulatory action of IL-1 on collagen synthesis might therefore be obscured if PGE_2 accumulated in the medium. Any experimental situation favouring accumulation of substantial amounts of PGE_2 in the

Abbreviations used: IL-1, interleukin-1; PGE_2, prostaglandin E_2; Indo, indomethacin; ASA, acetylsalicylic acid; BW 755 C, 3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline hydrochloride; PAGE, polyacrylamide-gel electrophoresis; DME, Dulbecco's modification of Eagle's medium; FCS, fetal-calf serum; MCF, mononuclear-cell factor.

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culture medium would result in depression of collagen synthesis by IL-1, whereas a stimulatory effect could be revealed by preventing the synthesis of PGE_2 with a cyclo-oxygenase inhibitor or limiting its level by changing the culture medium.

Here we report the effects of homogeneous porcine IL-1 α on collagen production by adherent synovial cells under various experimental conditions. We conclude that IL-1 alone decreases collagen synthesis, mainly as a result of enhanced PGE_2 release. By contrast, the effect is suppressed, and sometimes reversed (depending on the cell strain), when the cultures are treated with Indo to prevent prostaglandin production. The decrease in collagen production mainly affects type I collagen, type III being almost unaltered. In contrast with the production rate for type I collagen in the medium, the steady-state level of mRNA for type I procollagen was increased by exposure to IL-1, suggesting that post-transcriptional controls could be involved in the mechanism by which IL-1 modulates collagen synthesis.

MATERIALS AND METHODS

Materials

Porcine IL-1 α (acidic form of IL-1 of pI 5) from peripheral-blood leucocytes was purified to homogeneity as described elsewhere (Saklatvala *et al.*, 1985). Dulbecco's modification of Eagle's culture medium (DME medium) and fetal-calf serum (FCS) were obtained from Serva (Heidelberg, Germany). Collagenase and trypsin used for tissue dissociation were from Sigma (St. Louis, MO, U.S.A.). Highly purified bacterial collagenase (ABC, form III) was purchased from Advance Biofactures Corp. (Lynbrook, NY, U.S.A.). Pepsin (three-times-crystallized) was supplied by Calbiochem. [4,5-^{3}H]Proline (32 Ci/mmol; 1.18 TBq/mmol) came from CEA (Saclay, France). Standard [5,6,8,11,12,14,15 (n)-^{3}H]PGE_2 (200 Ci/mmol; 7.37 TBq/mmol) was from New England Nuclear (Boston, MA, U.S.A.). Anti-PGE_2 serum was obtained from the Institut Pasteur (Paris, France). All other chemicals were analytical reagents from Sigma.

Synovial cell culture

Culture of human adherent rheumatoid synovial cells were prepared by sequential enzymic digestion (Dayer *et al.*, 1976) and maintained in DME medium containing 10% FCS (heat-inactivated for 30 min at 56 °C), penicillin (100 i.u./ml), streptomycin (100 μg/ml) and fungizone (2.5 μg/ml), at 37 °C in CO_2/air (1:19). The medium was changed at 2–3-day intervals. In the experiments to be described, these cells were generally used between the first and third passage and plated in 2 cm^2 culture wells (Falcon 3047) at 10^5 cells/well in the same medium as that described above. The cultures were grown to confluency before experimental use.

Collagen labelling and assay

Confluent cells in 24-well plates were incubated with or without IL-1, in DME medium containing 10% FCS and supplemented with sodium ascorbate (50 μg/ml) and β-aminopropionitrile (50 μg/ml). [^{3}H]Proline (5 μCi/ml) was added, and the cultures were pulsed for 24 h. At the end of incubation, the culture medium was collected and the labelled collagen was assayed with pure bacterial collagenase (Advance Biofactures Corp.) as described by Peterkofsky & Diegelmann (1971). We previously showed that approx. 90–95% of the total collagen produced was recovered in the medium when synovial cells are incubated in presence of ascorbate and β-aminopropionitrile, so that we only studied this fraction in our experiments. Non-collagen protein was estimated by subtracting collagenase-digestible radioactivity from the total radioactivity incorporated into protein.

Analysis of collagen by gel electrophoresis

Cells were incubated for 24 h with 20 μCi of [^{3}H]-proline/ml. Then the medium was collected and the cell layer rinsed with PBS, which was added to the first extract. The samples were dialysed against 1 mM-ammonium acetate for 72 h and freeze-dried. After dilution in 1 ml of 0.15 M-NaCl, a mixture composed of 125 μl of 5 M-acetic acid, 125 μl of pepsin (1 mg/ml) and 50 μl of 1% (w/v) of L-proline was added and the samples incubated overnight at 4 °C. After freeze-drying, the mixtures were dissolved in 600 μl of electrophoresis buffer [0.025 M-Tris/glycine, pH 8.3, containing 0.1% (w/v) SDS] and analysed by SDS/PAGE in the presence of 2 M-urea (O'Farrell, 1975) without or with reduction (Sykes *et al.*, 1976). The labelled collagens were identified by fluorography (Bonner & Laskey, 1974) and quantified by densitometry.

Radioimmunoassay of collagens

A radioimmunoassay of type I and type III collagens was carried out by the method of Magloire *et al.* (1986). Type-specific antibodies were raised in rabbits and isolated by affinity chromatography on the corresponding collagen type. Antigens were radioiodinated by the Chloramine-T method. To determine the immunoreactivity of labelled collagen and the titre of the collagen antibodies, binding of ^{125}I-collagen was carried out with serial dilutions of the collagen antibody; to 0.1 ml of various dilutions of antibody in 0.5% normal rabbit serum diluted in PBS/Tween, 0.1 ml of ^{125}I-collagen and 0.1 ml of PBS/Tween were added. After 24 h incubation at 4 °C the bound fraction was separated from the free by precipitation with 0.1 ml of goat antiserum to rabbit IgG and 1 ml of 1.5% (w/v) poly(ethylene glycol) in PBS/Tween, and by centrifugation. Non-specific binding was measured by replacing the collagen antibody with normal rabbit serum. To evaluate the released type I and type III collagens in the culture medium, 0.1 ml of optimally diluted anti-(type I collagen) or anti-(type III collagen) antibody (which gave between 30 and 60% binding) and 0.1 ml of PBS/Tween were incubated at concentrations ranging from 40 to 5000 μg/ml or with 0.1 ml of culture medium; 0.1 ml of type I or type III ^{125}I-collagen was then added and the reaction continued as described above.

Quantification of collagen-gene expression

Dot-blot hybridization to total cellular RNA was used to quantify gene expression at the mRNA level. The data were expressed per flask, since control cell counting previously showed that there was no appreciable difference between control and treated cells. Extraction of total RNA was carried out by the guanidine thiocyanate method (Cathala *et al.*, 1983). The extracts were diluted at various concentrations (0.078–10 μg) of total RNA

with a solution of 6.15 M-formaldehyde/10 × SSC (1 × SSC is 0.15 M-NaCl/0.015 M-sodium citrate). RNA was denatured at 65 °C for 15 min and applied to nitrocellulose sheets (Schleicher and Schüell; BA 85; 0.45 μm pore size) by employing a Minifold apparatus (Schleicher and Schüell). Then nitrocellulose was baked at 80 °C for 90 min under vacuum. Hybridization of the dots was carried out with two probes labelled with ^{32}P by nick translation; α_2(I) mRNA was detected with the 4.0-kb *Eco*RI fragment of HpC_1, an exon-containing region of an α_2(I) genomic clone (Dalgleish *et al.*, 1982), kindly provided by Dr. R. G. Crystal (National Institutes of Health, Bethesda, MD, U.S.A.), α_1(III) mRNA was quantified by using the pHFS3 clone prepared by Sandberg & Vuorio (1987) and kindly given by Dr. E. Vuorio (Turku, Finland). This latter was constructed by cloning a 700-bp *Xho*I/*Eco*R1 fragment of human proα1(III) collagen cDNA in pUC8 between the single *Sal*I and *Eco*R1 sites. Quantification of the dots was performed by scanning the autoradiograms with a densitometer and expressing the results in arbitrary units.

Assay of PGE_2

PGE_2 was assayed in the culture medium by specific radioimmunoassay (Dray *et al.*, 1975) with antiserum from Institut Pasteur Production (Marnes la Coquette, France).

Addition of drugs

Indo and ASA were dissolved in a small volume of ethanol and then diluted with the culture medium to the required concentrations. The amount of ethanol present in the cultures had negligible effect on collagen synthesis. BW 755 C (Wellcome Research Laboratories) was directly dissolved in the medium.

RESULTS

Effect of porcine IL-1 on collagen and non-collagen protein synthesis

Fig. 1 illustrates the effect of 0.05, 0.5 and 5 ng of porcine IL-1 α/ml on the accumulation of collagen and non-collagenous protein in the culture medium during a 24 h incubation. IL-1 diminished the collagen synthesis in a dose-dependent manner, the inhibition level reaching about 40 % for the highest concentration used (5 ng/ml). Table 1 shows that the inhibition was not due to impaired secretion of collagen, since we did not observe any significant variation of the relative distribution between media and cell layers. In contrast, the amount of non-collagenous protein produced was not significantly altered. Since IL-1 at 5 ng/ml markedly diminished collagen production without decreasing synthesis of non-collagenous protein, it was decided to use this concentration for most of the experiments.

Effect of pretreatment with IL-1 on collagen synthesis

In order to determine whether the decrease in collagen synthesis was dependent on continuous exposure to IL-1, two types of experimental conditions were used. In the first one, cultures in DMEM + 10 % FCS were incubated for 48 h with IL-1 (5 ng/ml). Then the medium was discarded and fresh medium containing sodium ascorbate (50 μg/ml), β-aminopropionitrile (50 μg/ml), IL-1 (5 ng/ml) and [^{3}H]proline (5 μCi/ml) was added for a further 24 h. The second procedure was similar, except that the IL-1 was omitted during the 24 h labelling period. The degree of inhibition of collagen synthesis at the concentrations tested was the same for both procedures (results not shown).

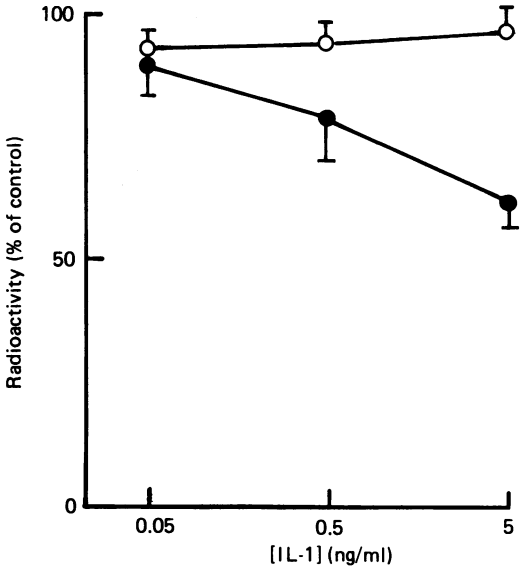

Fig. 1. Dose-dependence of the action of IL-1 on collagen synthesis

The effect of porcine IL-1 α was assayed at increasing concentrations. Confluent synovial cells were incubated for 24 h as described in the Materials and methods section. Collagen radioactivity was estimated as d.p.m. in collagenase-digestible material present in the medium (●). Non-collagen protein represents the amount of radioactivity remaining after collagenase digestion (○). Values are expressed as percentages of controls (collagen, 8500 ± 656 d.p.m.; non-collagen protein, 23448 ± 912 d.p.m.). The bars represent S.E.M. values for triplicate assays.

Table 1. Distribution of collagen between cell layers and media

Confluent synovial-cell cultures in 10 cm² Petri dishes were incubated for 24 h with and without IL-1 (5 ng/ml) in DME medium containing 10 % FCS, sodium ascorbate (50 μg/ml), β-aminopropionitrile (50 μg/ml) and [^{3}H]-proline (2 μCi/ml). Labelled collagen in the media and cell layers was assayed as collagenase-digestible protein. All experiments were performed in triplicate, and the values shown are the average of three determinations, results of which differed from each other by less than 10 %.

Culture conditions	Collagen radioactivity (d.p.m./dish)	Collagen (% of total)
Controls		
Media	20291	90.0
Cell layers	2256	10.0
Total	22547	
IL-1-treated		
Media	9630	91.5
Cell layers	891	8.5
Total	10521	

Table 2. Effect of cyclo-oxygenase inhibitors on collagen and PGE_2 production

Synovial cells (plated at 2×10^5 cells/ml) were grown to confluency in 24-well plates. Incubation was carried out in 0.5 ml of DMEM, containing 10% (v/v) fetal-calf serum, supplemented with sodium ascorbate (50 μg/ml), β-aminopropionitrile (50 μg/ml) in presence or absence of IL-1 (5 ng/ml). [^{3}H]Proline (2 μCi/ml) was added in the series used for collagen labelling, the other one being employed for PGE_2 assay. Measurements were performed on the medium described in the Materials and methods section. Results are expressed as means (±S.E.M.) of triplicate determinations. Statistical analysis (Student's t test) was used to compare IL-1-treated samples with corresponding controls containing inhibitors alone; $^{**}P < 0.01$ versus respective control.

	Collagen		PGE_2	
Sample	(d.p.m./well)	(% of control)	(pmol/ml)	(% of control)
Control	6378±360	100	2440±331	100
IL-1	2870±165**	45±2.6	23521±454	965±19
Indo	5208±390	81.7±6.1	199±4	8.2±0.2
Indo+IL-1	5176±286	81.2±4.5	63±9	2.6±0.4
ASA	5437±375	85.2±5.9	239±39	9.8±1.6
ASA+IL-1	4761±201	74.6±3.2	59±11	2.4±0.5
BW 755 C	4228±70	66.3±1.1	73±21	3±0.9
BW 755 C+IL-1	4893±371	45.2±5.8	486±8	2.3±1.1

Table 3. Comparative effects of indomethacin, PGE_2 and dibutyryl cyclic AMP on stimulated (+IL-1) and unstimulated (−IL-1) synovial cells

Confluent synovial cells were incubated under same conditions as those described for Table 2. Indo was added at 10 μM, PGE_2 at 0.3 μM and dibutyryl cyclic AMP (dbcAMP) at 5 μM. Collagen radioactivity (d.p.m.) is expressed as the mean (±S.E.M.) ($n = 3$) and as percentages of controls. Statistical differences between IL-1-treated samples and respective controls were analysed by Student's t test; $^{*}P < 0.05$ versus respective control.

	Collagen	
Sample	(d.p.m./well)	(% of control)
Control	10030±675	100
IL-1	5516±211*	55±2.1
Indo	10884±784	108.5±7.8
Indo+IL-1	12291±934	122.5±9.3
Indo+PGE_2	8249±438	82.2±4.3
Indo+PGE_2+IL-1	5717±473*	57±4.7
Indo+dbcAMP	9077±548	90.5±5.5
Indo+dbcAMP+IL-1	5416±817*	54±8.1

Effect of cyclo-oxygenase inhibitors

Since IL-1 stimulates arachidonic acid release, with subsequent formation of active metabolites (mainly PGE_2), it was of interest to determine the effect of inhibitors of the cyclo-oxygenase pathway on the amount of collagen produced. Levels of PGE_2 and collagen secreted into the culture medium were measured in parallel. The results, summarized in Table 2, show that all the cyclo-oxygenase inhibitors used (Indo, ASA and BW 755 C) suppressed the ihibitory effect of IL-1 on collagen synthesis, since the values obtained with the combination of inhibitor plus IL-1 were similar to those observed in the presence of inhibitor alone ($P < 0.05$). At the same time, PGE_2 release, which was stimulated by IL-1 as expected (almost 10-fold), decreased to very low levels (2–8% of the controls) as a result of cyclo-oxygenase inhibition. These data suggest that IL-1 exerts its inhibitory action on collagen synthesis by a mechanism which may be partially dependent on PG production.

Action of exogenous PGE_2 and dibutyryl cyclic AMP in association with Indo

Since it has been shown that PGE_2 stimulates adenylate cyclase in various cells, and that increases of intracellular cyclic AMP may, in turn, diminish collagen synthesis (Baum *et al.*, 1978, 1980; Clark *et al.*, 1982), we studied the effect of exogenous PGE_2 and dibutyryl cyclic AMP as a complement to the preceding experiments. As Table 3 shows, the simultaneous addition of both Indo (10 μM) and PGE_2 (100 ng/ml) to the cultures induced a slight inhibition of collagen synthesis compared with the value for Indo alone ($P < 0.1$). Furthermore, in presence of Indo and PGE_2, IL-1 produced a greater decrease, similar in extent to that observed for IL-1 alone. Approximately the same pattern has been obtained with cyclic AMP. Taken together, these results suggest that cyclic AMP formed by PGE_2-activated adenylate cyclase plays some part in the inhibition of collagen synthesis under the influence of IL-1.

Quantification of type I and type III collagens by radioimmunoassay

In order to determine the effect of IL-1 on the proportion of different types of collagen produced, radioimmunoassays were performed on the medium using antisera to type I and type III collagens. Fig. 2 depicts the results obtained with media from synovial cells incubated alone or with IL-1, with or without inhibitors of cyclo-oxygenase. As the control results show, these synovial cells produced almost equal amounts of type III and type I collagen. IL-1 decreased the level of collagen type I without affecting that of type III. The inhibitors of cyclo-oxygenase pathway alone (Indo, BW 755 C and ASA) generally induced an increase of collagen synthesis, the extent of which varied according (probably) to their efficiency in blocking PG production. In the presence of such inhibitors, IL-1 still caused a small decrease in the production of collagen type I compared with that in cultures exposed to inhibitors

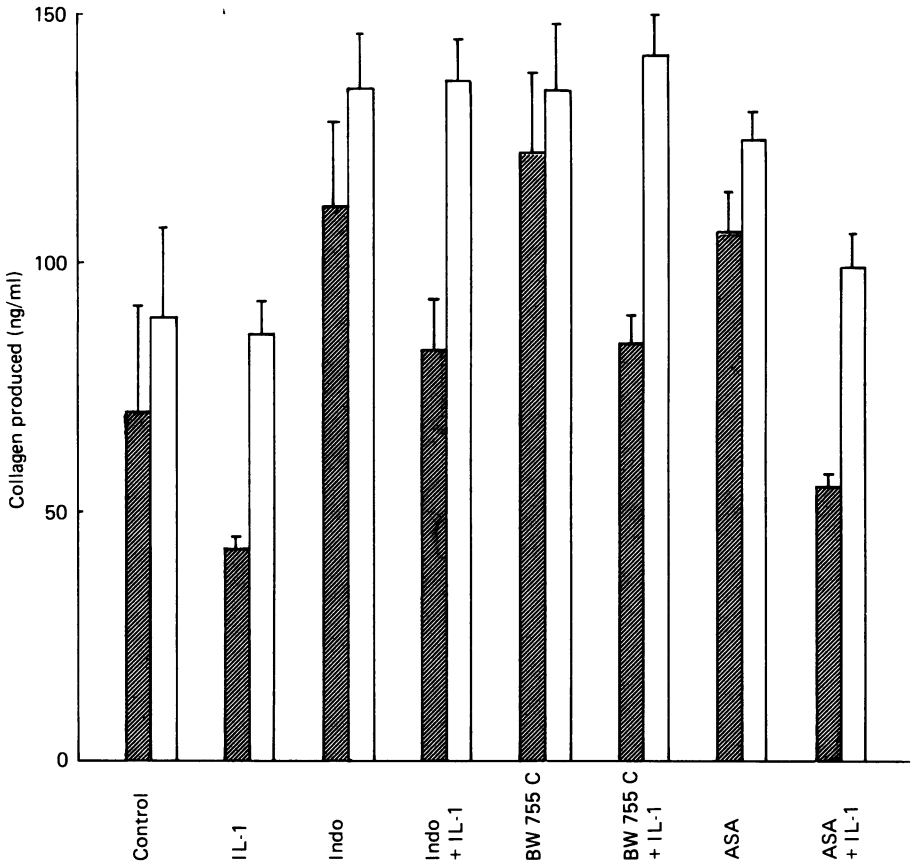

Fig. 2. Radioimmunoassay of type I and type III collagens produced by IL-1-treated synovial cells in presence of inhibitors of cyclo-oxygenase

Confluent synovial cells were incubated for 24 h with different inhibitors (10 μM), with or without IL-1 (5 ng/ml). Type I (▨) and type III (□) collagens were assayed in the culture media as described in the Materials and methods section and expressed as mean value ± S.E.M. ($n = 3$).

alone, whereas type III collagen remained relatively unaffected. Therefore, in our experiments, radioimmunoassays revealed a decrease in type I collagen under the influence of IL-1, even in the presence of inhibitors of PG synthesis, an effect which was not detectable at the level of total collagen production (Table 2).

Analysis of radiolabelled collagens by SDS/PAGE

In order to determine the ratio of type III to type I collagens, we have also used SDS/PAGE as an alternative to radioimmunoassay. The pattern of collagens synthesized, as resolved under non-reducing and reducing conditions, is shown in Fig. 3. All samples contained the same amount of total protein radioactivity (47000 c.p.m.). The amount of type I collagen in the cultures exposed to IL-1 was estimated, from scanning the 'reduced' electrophoretograms, to be approx. 26.0% of the control value. The level of type III collagen was also decreased in this experiment, albeit to a lesser extent: 44.3% of the control. Indo-treated cultures displayed a pattern very similar to that of controls. In the presence of both indomethacin and IL-1, the level of collagen type I was still decreased (48.4% of the value for Indo alone), but the amount of type III collagen remained unchanged. Finally, the percentages of type III collagen calculated from the densitometric scanning of the 'reduced' electrophoretograms were 17.3% for the controls, 25.9% for IL-1-treated cultures, 13.0% for Indo alone and 21.2% for Indo plus IL-1. These results are in agreement with those obtained from radioimmunoassay, which also showed an increase of the percentage of type III collagen upon IL-1 treatment.

Steady-state levels of mRNA for type I and type III procollagens

To determine whether exposure of synovial cells to IL-1, in the presence or absence of Indo, could alter the levels of procollagen mRNAs, total RNA was extracted from cultures incubated for 24 h and equal amounts of each preparation were analysed by dot-blot hybridization. The level of collagens actually present in the culture medium in these experiments was also measured. As seen in Fig. 4, which shows a representative experiment, IL-1 alone caused a slight increase in mRNA of type I procollagen. Indo markedly increased the amount of mRNA, whereas IL-1, when added with the drug, gave a slightly decreased value compared with Indo alone in that cell strain. However, we found some cell strains which responded with a 2-fold increase in the type I procollagen mRNA level when IL-1 was added together with Indo (Fig. 5). In contrast with type I procollagen mRNA, the level of mRNA for type III procollagen was slightly diminished by IL-1, and the amount of type III procollagen mRNA in Indo-treated cells was rather

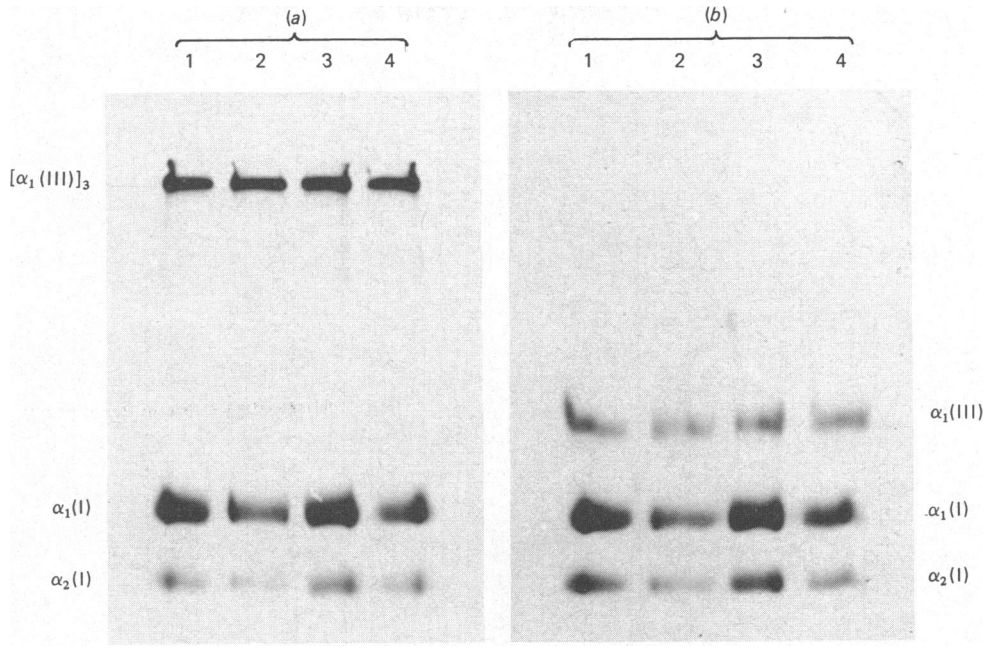

Fig. 3. Fluorogram of SDS/PAGE of collagens from synovial cells incubated with or without IL-1

Confluent synovial cells were treated for 24 h with or without (control) IL-1 (5 ng/ml) in the presence of [^{3}H]proline (20 μCi/ml). Labelled medium collagens were prepared as described in the Materials and methods section and analysed by SDS/6%-PAGE without (*a*) or with (*b*) reduction (interrupted electrophoresis). The deposits correspond to the same amount of total protein radioactivity. The positions of labelled bands were determined by fluorography. Lane 1, controls; lane 2, IL-1; lane 3, 10 μM-Indo; lane 4, 10 μM-Indo + IL-1.

similar to that of the control (Fig. 5). However, in the presence of the drug, IL-1 stimulated to a small extent the type III procollagen mRNA level (Figs. 4 and 5).

Comparison between the dot-blots and the radioimmunoassays of type I collagens present in the culture medium clearly revealed that type I collagen production did not correlate with the steady-state mRNA levels. Elevation of type I procollagen mRNA induced by IL-1 was associated with a decrease in the quantity of collagen released into the medium (Figs. 4 and 5). In contrast with type I collagen, only slight changes were registered in the amount of type III collagen under IL-1 treatment, and a better correlation was observed with the corresponding mRNA. From these data we can conclude that post-transcriptional mechanisms may counteract an increased level of type I procollagen mRNA caused by IL-1, resulting in a decrease in the amount of type I collagen produced by the cells.

DISCUSSION

There are conflicting reports in the literature concerning the effect of IL-1 on collagen synthesis in synovial cells. Krane *et al.* (1985) first demonstrated that a partially purified mononuclear-cell factor (MCF), later shown to be IL-1, could stimulate the collagen production of cultured rheumatoid synovial cells. On the other hand, we reported that a similar MCF preparation inhibited collagen synthesis (Pujol *et al.*, 1985). This apparent discrepancy suggested that modulation of collagen production by IL-1 may depend on a number of variable factors. For example, IL-1 elicits a marked rise in release of PGE_2 (Dayer *et al.*, 1979) which, in turn, may be responsible for the diminution in collagen synthesis (Baum *et al.*, 1978, 1980; Clark *et al.*, 1982). It should be noted that the most significant increase in collagen production observed by Krane *et al.* (1985) was seen in cultures treated with Indo to block PG synthesis, and we also found that this drug reversed the inhibition of collagen synthesis caused by IL-1 in our system (Pujol *et al.*, 1985). However, several other mechanisms could contribute to the effect of IL-1 on collagen synthesis (Freundlich *et al.*, 1986) and may complicate the evaluation of the primary effects of the monokine.

In an attempt to understand these mechanisms, we have studied the influence of pure IL-1 α on the production of collagen by rheumatoid synovial cells under different experimental conditions. One problem of such an investigation is that cultures of adherent synovial cells do not consist of a homogeneous population. Four main types of cells can be found after 24 h in culture: stellate cells, fibroblastic cells, macrophage-like cells and round monocytes (Heino *et al.*, 1987). We used the cultures at passage 2 or 3 which comprised stellate, but mainly fibroblastic, cells. Nevertheless, the relative proportion of these cellular types and the magnitude of response to IL-1 varied with cell strain.

In our experiments, the amount of radioactivity incorporated into collagen, estimated as collagenase-digestible material, was clearly decreased, in a dose-dependent manner, by a 24 h treatment with IL-1 α. However, the use of clostridial collagenase to quantify

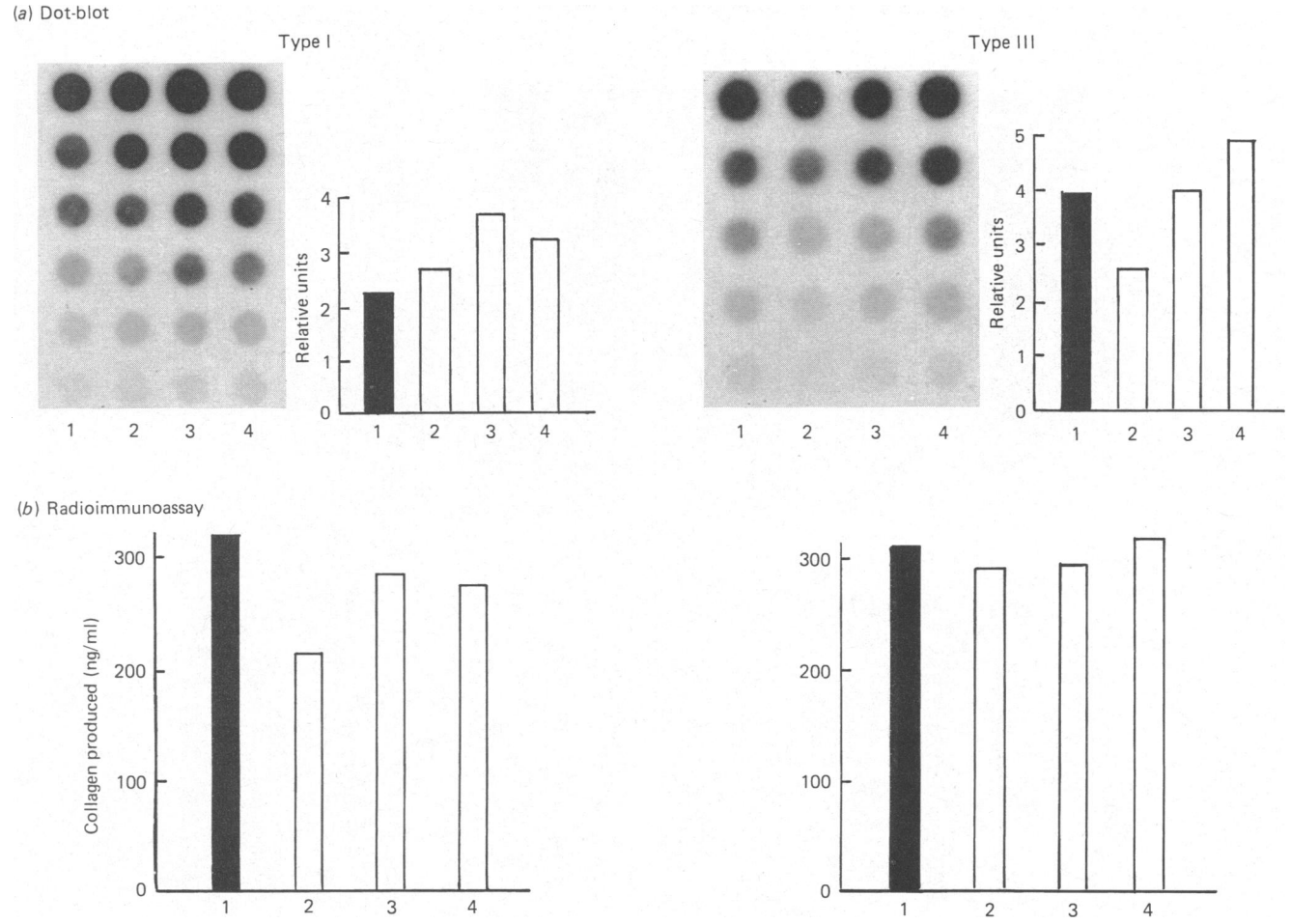

Fig. 4. Comparison between the steady-state levels of type I and type III collagen mRNAs and the corresponding amounts of collagens produced in the medium

Confluent synovial cells were treated for 24 h with IL-1 (5 ng/ml), or without, in the presence or absence of 10 μM-Indo. The media were then collected for evaluation of type I and type III collagens by radioimmunoassay. Total RNA was extracted from the cells as described in the Materials and methods section. (*a*) Steady-state levels of mRNAs. Equal quantities of RNA were applied to nitrocellulose filters and hybridized with ^{32}P-labelled cDNA probes for type I and type III collagen mRNAs. Fluorograms of the dots and their corresponding scannings are shown. (*b*) Radioimmunoassay of type I and type III collagens in the medium. The radioimmunoassays were performed on media samples in parallel with the mRNA analyses. 1, Controls; 2, IL-1; 3, 10 μM-Indo; 4, IL-1 + 10 μM-Indo.

collagen production in the present study may be questioned, since it assumes that IL-1 does not alter the cellular uptake of proline, thereby changing the specific radioactivity of collagen synthesized. This could lead to errors in the actual amount of collagen produced in presence of IL-1. For this reason we also used a radioimmunoassay to measure collagen production. We constantly observed a smaller apparent inhibition of collagen synthesis by IL-1 when using radioimmunoassay than with the collagenase method. The effect of IL-1 was specific for collagen, since no significant alteration could be observed in the amount of non-collagenous protein. These results confirm our previous findings obtained with partially purified MCF (Pujol *et al.*, 1985), but are in apparent contradiction with those of Krane *et al.* (1985), who reported a stimulatory effect of IL-1 on collagen synthesis in rheumatoid synovial cells. However, it should be noted that these authors used a different procedure, which consisted of incubating cells for 48 h with MCF in the presence of 10% serum, then labelling the cultures for 24 h in the absence of serum and MCF. It is likely that this protocol minimized the amount of PGE_2 released and led to a stimulation of, instead of a decrease in, collagen synthesis.

The addition of drugs inhibiting PG synthesis to the cultures sometimes increased the production of collagen compared with the controls, the extent of this stimulatory effect depending on the cell strain. In presence of such inhibitors, the depressive effect exerted by IL-1 was generally decreased or abolished, suggesting that the mechanism responsible for the decrease in collagen synthesis was largely PG-dependent. Moreover, we have been able to reproduce to some extent the IL-1-induced inhibition by adding PGE_2 to Indo-treated cultures. Taken together, the results support the possibility that the inhibitory action of IL-1 on collagen synthesis is in part due to the action of PG.

A high rate of synthesis of type III collagen relative to type I by synovial cells has already been reported by Goldberg *et al.* (1980) and Parrott *et al.* (1980). However,

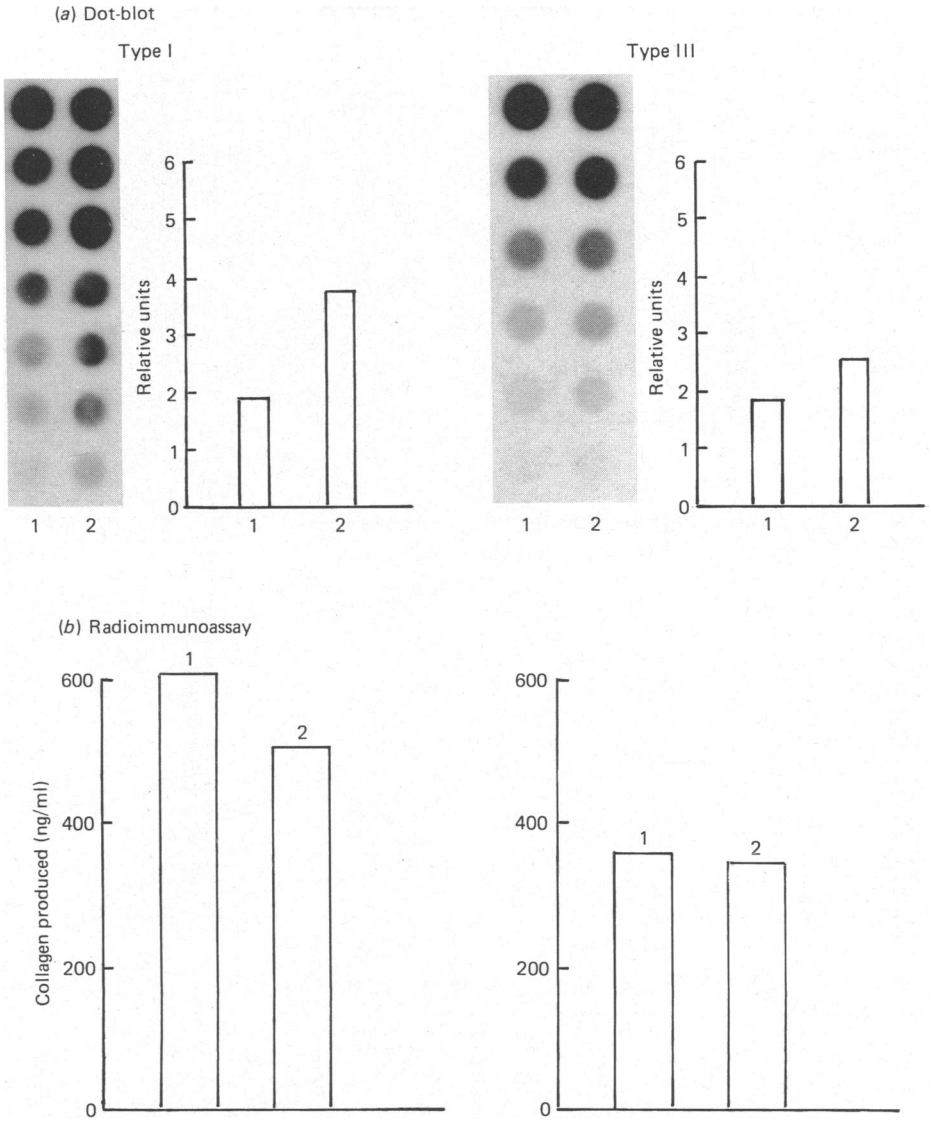

Fig. 5. Representative example of indomethacin-treated synoviocyte strain responding to IL-1 by a great increase of the level of collagen type I mRNA

The procedure was identical with that of Fig. 4. (*a*) Steady-state levels of mRNAs; (*b*) radioimmunoassay of type I and type III collagens. 1, Indo; 2, Indo + IL-1.

we found striking variations in the relative proportion of type I and type III collagen in our cultures, depending on the strain. Whatever the proportion of the two genetic types, our data show that principally it is the amount of type I collagen that is diminished by IL-1 treatment. As a consequence, the ratio of type III to type I collagens released into the extracellular matrix is elevated. We do not know at present whether this IL-1-induced change in collagen distribution observed *in vitro* could be effective *in vivo* and have pathophysiological significance.

The biosynthesis of collagen can be potentially regulated at transcriptional and post-transcriptional steps. We therefore measured collagen type I and type III mRNA levels in several synoviocyte strains to determine whether decreased collagen synthesis in IL-1-treated cells was reflected in the steady-state levels of their corresponding mRNAs. The data obtained clearly indicated that the total amount of type I collagen mRNA did not correlate with the quantity of collagen released into the medium over the same period. We generally observed a stimulation of type I collagen mRNA by IL-1, whereas the level of collagen secreted, as estimated by radioimmunoassay, was decreased. Our results also demonstrated that Indo is a potent stimulator of procollagen type I mRNA.

The amount of type III collagen secreted into the medium was only slightly affected by exposure of cells to IL-1, and we generally observed a better correlation with the level of mRNA than for type I collagen.

Our findings suggest that both transcriptional and post-transcriptional mechanisms may be involved in the modulation of collagen synthesis by IL-1. Although many laboratories have reported that the steady-state type I collagen mRNA levels are a major factor in determination of the amount of type I collagen synthesis (Adams *et al.*, 1977; Moen *et al.*, 1979; Sobel *et al.*, 1981; Rowe & Schwarz, 1983), several recent studies have described situations where the amount of type I collagen

protein synthesized does not correlate with the steady-state RNA levels. For example, the amount of type I collagen mRNA in human fetal lung fibroblasts remains relatively constant whether the cells are confluent or proliferating, whereas the rate of protein synthesis is higher in proliferating cells (Tolstoshev *et al.*, 1981*a*). Similarly, type I collagen mRNA does not significantly change in the skin during sheep embryogenesis; however, the synthetic rate of collagen decreases at the final period of embryogenesis (Tolstoshev *et al.*, 1981*b*). One explanation for our results could be that the translatability of type I procollagen mRNA of IL-1-treated synovial cells is decreased compared with the controls. Further analysis of this parameter will be required before any conclusion can be reached. However, in view of the results obtained by others (Krane *et al.*, 1985; Kähäri *et al.*, 1987) showing that IL-1 can stimulate collagen synthesis under some experimental conditions, together with our data on Indo-treated cultures, it is likely that an additional control mechanism involving PGE_2 could exist at a post-translational level. Baum *et al.* (1980) demonstrated that the stimulation of cyclic AMP by PGE_2 was accompanied by an increase in intracellular collagen degradation. Therefore, we suggest that the IL-1-stimulated increase of type I collagen mRNA in synovial cells could result in enhanced synthesis of procollagen, but that this may be counterbalanced by an elevated rate of intracellular degradation due to the action of PGE_2. So the rate of collagen release into the medium could be the net result of a stimulation of transcription and a negative feedback acting at a post-transcriptional stage. Studies on the early events in the effect of IL-1 on collagen synthesis, as well as measurement of intracellular degradation of collagen are needed to provide further information on the mechanisms by which IL-1 may regulate production of collagen.

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