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EFFECTS OF PROSTAGLANDIN E_2, INDOMETHACIN, TRIFLUOPERAZINE AND DRUGS AFFECTING THE CYTOSKELETON ON COLLAGENASE PRODUCTION BY CULTURED ADHERENT RHEUMATOID SYNOVIAL CELLS

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Abstract—Cultured adherent rheumatoid synovial cells with fibroblast properties release large amounts of collagenase and prostaglandin E_2 (PGE_2) into the medium. With age in culture and passage of the cells, the levels of collagenase and PGE_2 decrease, but can be increased by a factor (MCF; mononuclear cell factor) released by cultured human blood monocyte-macrophages. The magnitude of the stimulation varies with different synovial cell strains. To determine some of the mechanisms which regulate the collagenase response, synovial cells were exposed to a cyclooxygenase inhibitor (indomethacin) and substances which alter the cytoskeleton (cytochalasin B or colchicine) or interact with Ca^{2+}·calmodulin (trifluoperazine). The collagenase response was retained even when PGE_2 synthesis was totally blocked with indomethacin. The collagenase response, however, was blunted at high indomethacin concentrations ($> 10\ \mu M$) and paradoxically augmented at lower indomethacin concentrations ($0.001\ \mu M$). In some synovial cell strains, the blunting effect of $10\ \mu M$ indomethacin was reversed by the addition of low concentrations of exogenous PGE_2 (10 ng/ml). Preincubation of synovial cells for 1 or 24 hr with colchicine or cytochalasin B ($1–10\ \mu M$) resulted in an augmented collagenase and PGE_2 response to MCF. Cells preincubated or incubated with $1–50\ \mu M$ trifluoperazine, a phenothiazine, also augmented collagenase stimulation by MCF, but, in contrast to colchicine or cytochalasin B, trifluoperazine suppressed the PGE_2 response. Thus, although PGE_2 and collagenase production by synovial cells may be dissociated, altering ambient PGE_2 levels affected basal collagenase production and modulated the collagenase response to MCF.

The destruction of juxtasynovial bone, cartilage and tendons is a characteristic of inflammatory joint disease, such as rheumatoid arthritis. The release of collagenase and prostaglandins, such as prostaglandin E_2 (PGE_2), by cells in the adjacent synovial pannus is considered to play a role in the pathogenesis of the lesion [1, 2]. Animal collagenases act on types I and III collagens at a similar rate but cleave type II collagen at a slower rate. These enzymes, which act at neutral pH, produce specific cleavages, across the three chains of the helical collagen molecules at a point 3/4 the distance from the amino terminus. The cleaved fragments are then solubilized from the fibril. The collagenases secreted by several different animal tissues, including rheumatoid synovial fragments in culture, can utilize the interstitial collagens found in the joint as substrates [1]. Products of arachidonic acid metabolism, such as PGE_2, in addition to their potential role in accelerating bone resorption, may be pro-inflammatory (alter vascular permeability and produce pain) or anti-inflammatory (impair lymphocyte reactivity, decrease lymphokine production) [3]. These topics have been reviewed recently [4]. In a previous report, we demonstrated that adherent "stellate" synovial cells in primary culture produce large amounts of collagenase and PGE_2. After passage and time the basal levels of collagenase and PGE_2 decrease, and the stellate morphology tends to diminish. At this stage, however, the synovial cells can be stimulated by a factor (MCF; mononuclear cell factor) released by blood monocytes to synthesize high levels of collagenase and PGE_2 [5–7]. Furthermore, the stellate aspect can be reinduced by MCF, mediated by increases in ambient PGE_2 concentrations [8]. MCF, which has an apparent molecular weight of 15,000 daltons by gel filtration, after various chromatographic steps, still stimulates synthesis of both collagenase and PGE_2 [9]. Purified MCF shares biological properties with interleukin 1 [10].

Although production of collagenase and PGE_2 can always be stimulated by MCF, the levels of these two products can be dissociated under certain conditions. In contrast to glucocorticoids, which block both collagenase and PGE_2 production, indomethacin, a cyclooxygenase inhibitor, always suppresses PGE_2 levels while variably affecting collagenase levels. Our objectives in the present report were to investigate possible mechanisms whereby collagenase and PGE_2 production are linked or dissociated in the synovial cell culture system.

It had been shown previously that the cAMP content of adherent synovial cells is increased by expos-

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ure to PGE_2 and that MCF in the presence of indomethacin potentiates this cAMP response to exogenous PGE_2 [11]. Thus, the influence of PGE_2 concentrations on collagenase production may, in part, be mediated by cAMP. Observations of this sort have been made in other systems, such as in murine peritoneal macrophages [12]. It has also been shown in other systems that compounds such as cytochalasin B, which bind to and dissaggregate microfilaments [13], or colchicine, which binds to tubulin and disrupts microtubules [14], also stimulate production of latent collagenase. The arrangement of the microtubules and microfilaments is also regulated by the adenylate cyclase system [15]. We therefore designed experiments to determine whether altering the state of the cytoskeleton prior to or during MCF stimulation modifies the MCF response as measured by collagenase and PGE_2. Furthermore, since it has been shown that calmodulin can stimulate phospholipase A_2 activity in isolated platelet membranes [16] and trifluoperazine has been found to inhibit that stimulation, we examined the effects of trifluoperazine in this system. We found that cytochalasin B and colchicine potentiated both the collagenase- and PGE_2-stimulating effects of MCF. Indomethacin, at relatively high concentrations, in some but not all synovial cell cultures blunted the collagenase responses which could then be restored by exogenous PGE_2. Trifluoperazine, in contrast, potentiated the effects of MCF on collagenase production but suppressed the increase in PGE_2 synthesis.

MATERIALS AND METHODS

Synovial cell culture. Cultures of adherent rheumatoid synovial cells were prepared and maintained as in previous reports [3]. In the experiments to be described, these cells were used between the first and fourth passage and plated in culture wells, 16 mm diameter (Costar, Data Packaging Corp., Cambridge, MA), at $5–10 \times 10^4$ cells per well in Dulbecco's modified Eagle's medium [DMEM; Grand Island Biological Co. (GIBCO), Grand Island, NY] with 10% fetal calf serum (FCS, Microbiological Associates, Bethesda, MD) and penicillin (10 units/ml), streptomycin (100 μg/ml) (GIBCO). Incubations were carried out for 72 hr after which media were removed and assayed for collagenase activity and PGE_2 concentration.

Mononuclear cell factor (MCF). Mononuclear cell factor was obtained from pooled medium conditioned by cultured human blood mononuclear cells as described previously [9, 11]. The material used in the experiments reported here was prepared according to the techniques previously described, by chromatography on Ultrogel AcA54 (LKB Instruments, Inc. Rockville, MD) followed by chromatography on DEAE 52 cellulose [9, 11].

Collagenase assay. For assays of collagenase activity, [^{14}C]glycine-labeled guinea pig skin collagen (sp. act. ~10,000 dpm/μg) was used. [^{14}C]Collagen was reconstituted as fibrils in 40 mM Tris–HCl, pH 7.5, 120 mM NaCl, 2.7 mM $CaCl_2$. One unit of collagenase activity is defined as the solubilization of 1 μg of reconstituted fibrils per min at 37°. Incubation varied from 1 to 24 hr depending upon the activity. All assays included a sample incubated with trypsin, 100 μg/ml, to control for the extent of denaturation of the collagen substrate. To detect activity in samples containing 10% FCS, samples were first activated with trypsin-TPCK (Worthington TRTPCK), final concentration 200 μg/ml, in 100 mM Tris–HCl, pH 7.4, 5 mM $CaCl_2$. After incubation for 10 min at 25°, soybean trypsin inhibitor (Worthington, SI) in the same buffer was added to give a final concentration of 500 μg/ml. An aliquot portion of this solution was then assayed on the [^{14}C]collagen fibrils, as described [3].

Prostaglandin assay. Prostaglandin concentrations were determined by radioimmunoassay utilizing an antiserum with specificity towards prostaglandin E, A and B [3] provided by Dr. L. Levine (Brandeis University, Waltham, MA). In previous reports, it had been shown by thin-layer chromatography that approximately 80% of the prostaglandin E + A + B was accounted for by PGE_2 and the remainder by PGE_1 [3].

Cell counting. After removing the medium, cells were trypsinized and counted using a Coulter counter model 2 (Coulter Electronics, Inc., Hialeah, FL) or a hemocytometer. PGE_2 was provided by Dr. J. Pike (Upjohn Co., Kalamazoo, MI) and prepared by diluting a stock solution of 1 mg/ml in absolute ethanol with buffer or DMEM, 10% FCS. Indomethacin (Merck Sharpe & Dohme, West Point, PA) was prepared by diluting a stock solution of 10 mM in ethanol with DMEM, 10% FCS. Cytochalasin B was obtained from the Aldrich Chemical Co. (Milwaukee, WI) and colchicine from the Sigma Chemical Co. (St. Louis, MO). Trifluoperazine dihydrochloride salt (Stelazine) was a gift of Dr. R. M. Myerson, Smith Kline & French Laboratories (Philadelphia, PA).

RESULTS

Collagenase and PGE_2 production by synovial cells in the presence of indomethacin. To determine the extent to which collagenase production is dependent upon PGE_2 levels, we examined twelve strains of synovial cells at the first passage when still producing high levels of collagenase and PGE_2 (Fig. 1). The absolute levels of collagenase in the absence of the drug ranged from 0.02 to 60.1 units/10^6 cells/day (mean, 7.57) and those of PGE_2 ranged from 0.89 to 522.5 ng/10^6 cells/day (mean 56.5). Since the absolute levels of collagenase and PGE_2 varied from one specimen to another, the effects of indomethacin are presented in Fig. 1 as a percentage of the levels in the absence of the drug. The mean concentration of indomethacin which resulted in 50% inhibition of PGE_2 synthesis in the twelve synovial cell strains was 0.001 μM.

In contrast, even at concentrations greater than 0.01 μM, indomethacin produced considerably less inhibition of collagenase production. Increases in collagenase levels above controls were observed at low concentrations of indomethacin (0.001 μM) in five of the twelve cultures, whose data was summarized in Fig. 1. Increasing the concentration of indomethacin to 1 μM markedly inhibited PGE_2 synthesis whereas

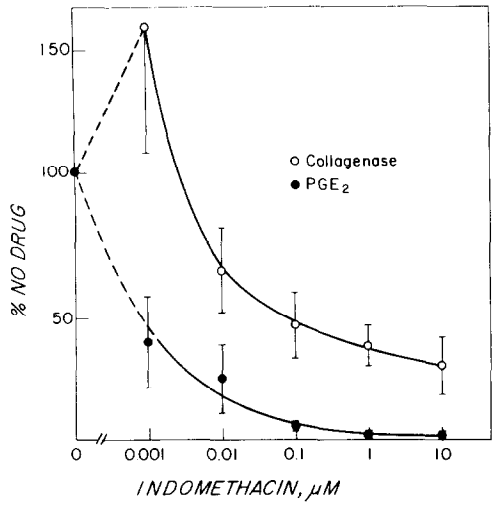

Fig. 1. Effect of indomethacin on collagenase and PGE_2 production by unstimulated adherent rheumatoid synovial cells. Synovial cell strains (N = 12) at the first passage were incubated for 3 days at 0.05×10^6 cells/well/0.5 ml DMEM, 10% FCS in the absence of indomethacin or the presence of various concentrations of indomethacin. Medium was then removed, and PGE_2 concentration (●) and collagenase activity (○) were determined. Values indicate percent activity ± S.E.M. in the absence of drug.

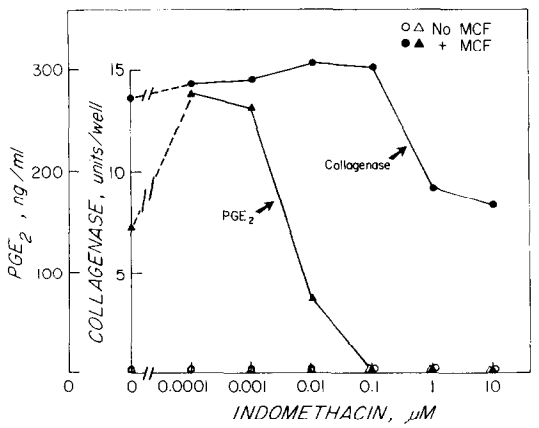

Fig. 2. Effect of indomethacin on collagenase and PGE_2 production by synovial cells with and without stimulation with mononuclear cell factor (MCF). One strain of synovial cells at the third passage was incubated under conditions similar to those described in Fig. 1. In this experiment cells were (●, ▲) or were not (○, △) stimulated with MCF. Medium was removed and assayed for collagenase activity (●, ○) or PGE_2 concentration (▲, △). Values represent means of duplicate wells.

collagenase production was inhibited only by a mean of 55%. Even at 1 μM indomethacin collagenase production by other strains of synovial cells (data not shown) was unchanged compared to the untreated cultures.

In older cultures at later passages where the cells usually required stimulation with MCF in order to obtain measurable collagenase and PGE_2 levels in the medium, a similar pattern was also observed. The results of the effects of different concentrations of indomethacin on collagenase and PGE_2 production in a representative culture are shown in Fig. 2. In this third passage culture, the levels of collagenase and PGE_2 were not measurable without MCF stimulation. When cells were stimulated with MCF, PGE_2 levels were 14 ng/ml and collagenase activity 6.5 units/well. Increases in PGE_2 at the lowest concentration of indomethacin tested (0.1 and 1 nM) were noted. However, at drug levels (1 μM) where PGE_2 production was nearly abolished, collagenase levels were unaffected.

In eleven other synovial cell cultures examined in the second to the fourth passage, incubation with MCF resulted in an increase in medium PGE_2 concentrations ranging from 2- to 452-fold and in collagenase activity ranging from 1.3- to 150-fold depending upon the sample (data not shown). The stimulation of PGE_2 synthesis in these cells exposed to MCF was inhibited by a mean of 97% (range 93–99%) in the presence of 10 μM indomethacin. In contrast, the mean inhibition of the MCF-stimulated collagenase production by indomethacin in these synovial cell strains stimulated with MCF was only 28% (range 0–90%). It should be noted that indomethacin had no effect at all on MCF-stimulated collagenase activity in four of these cell strains. The results demonstrate that collagenase production by individual specimens of synovial cells is variably affected by indomethacin. Attempts to determine the mechanisms for this variability were unsuccessful. Differences in morphology, density, or age and passage of the cultures were not determinants of the pattern of response to indomethacin.

Effect of exogenous PGE_2 on collagenase production. Since the synovial cell strains responded variably to indomethacin in the absence or presence of MCF, experiments were designed to test the possibilities that indomethacin could regulate collagenase production directly or indirectly through PGE_2 released into the medium. We chose cultures in which MCF-stimulated collagenase production was inhibited by indomethacin, as shown in Table 1. To examine the hypothesis that endogenous PGE_2 might be one of the modulators, we added exogenous PGE_2 to synovial cell cultures maintained in the absence or presence of indomethacin, stimulated or unstimulated by MCF. Furthermore, we attempted to determine the concentration of PGE_2 required to restore the blunting effect of indomethacin on MCF-treated cells. As shown in Table 1 in synovial strain number 1, 0.01 μg/ml of PGE_2 was sufficient to restore the collagenase response. Addition of exogenous PGE_2 to cell strain number 2, at 0.01 μg/ml, actually increased collagenase production above that observed in synovial cells exposed to MCF alone, suggesting a potentiation by exogenous PGE_2 of the MCF effect. This pattern of decrease in collagenase production by indomethacin in the presence of MCF was usually observed only at relatively high concentrations of indomethacin (10 μM or greater) and only in approximately one quarter of the cultures examined. In most instances, collagenase production was barely affected by the drug. In view of these experiments suggesting that collagenase levels can be modulated by PGE_2 levels, other compounds were also studied.

Table 1. Dose response of exogenous PGE_2 on collagenase production by synovial cells*

Cell strain number	Incubation conditions	Endogenous PGE_2 (ng/ml)	Collagenase (units/10^6 cells/day) With exogenous PGE_2 (μg/ml) 0	0.01	0.05	0.1	1.0
1	Control	8.54 ± 1.22	1.02 ± 0.03	0.56 ± 0.23		0.80 ± 0.21	0.31 ± 0.08
	MCF	58.40 ± 7.34	65.69 ± 3.93	87.20 ± 17.60		59.90 ± 5.50	54.10 ± 5.72
	MCF + indomethacin, 10 μM	3.8 ± 1.1	5.84 ± 0.98	72.06 ± 10.14		73.54 ± 14.09	53.92 ± 2.92
	Indomethacin, 10 μM	< 0.4	0.30 ± 0.28	2.03 ± 0.86		1.31 ± 0.70	0.73 ± 0.26
2	Control	3.03 ± 0.46	0.08 ± 0.03	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.36 ± 0.05
	MCF	349.28 ± 24.82	5.15 ± 0.40	2.99 ± 0.30	0.93 ± 0.10	0.89 ± 0.01	0.11 ± 0.01
	MCF + indomethacin, 10 μM	2.30 ± 0.60	2.38 ± 0.30	23.40 ± 2.40	6.62 ± 2.70	6.53 ± 1.30	2.05 ± 1.20
	Indomethacin, 10 μM	1.39 ± 0.12	0.12 ± 0.05	0.12 ± 0.06	0.13 ± 0.08	0.15 ± 0.05	0.14 ± 0.04

* Synovial cell strains at the fourth (number 1) or fifth passage (number 2) were incubated under the various conditions in medium alone (control), MCF, MCF plus indomethacin, or indomethacin. Buffer alone or exogenous PGE_2 at indicated concentrations was added at time zero, 24 hr and 48 hr to reach indicated concentrations. Values represent means + S.E.M. of triplicate wells.

Table 2. Effects of cytochalasin B and colchicine on MCF responses in synovial cells*

Preincubation 1 hr	Incubation 72 hr MCF	Collagenase (units/10^6 cells/3 days) Expt. 1	Expt. 2	PGE_2 ($ng/10^6$ cells/3 days) Expt. 1	Expt. 2
Control	−	0.01 ± 0.01	0.10 ± 0.05	250	725 ± 43
	+	17.30 ± 1.72	46.82 ± 3.93	6,197 ± 20	8,733 ± 634
Cytochalasin B, 1 μM	−	0.09 ± 0.04	0.13 ± 0.02	240 ± 2	135 ± 36
	+	30.68 ± 5.30	57.66 ± 5.44	7,792 ± 355	10,073 ± 390
Cytochalasin B, 0.1 μM	−	0.02 ± 0.01	0.20 ± 0.07	261 ± 2	136 ± 16
	+	26.33 ± 4.60	39.86 ± 2.05	8,140 ± 180	15,665 ± 2,730
Colchicine, 1 μM	−	1.09 ± 0.05	0.29 ± 0.19	209 ± 72	169 ± 17
	+	38.52 ± 3.05	44.76 ± 3.21	18,859 ± 448	16,998 ± 2,635
Colchicine, 0.1 μM	−	0.16 ± 0.05	0.14 ± 0.12	120 ± 10	167 ± 27
	+	50.22 ± 1.66	63.63 ± 0.27	2,989 ± 494	12,735 ± 1,540

* Two different strains (experiments 1 and 2) of synovial cells at the third passage were preincubated for 1 hr with medium alone (control), cytochalasin B or colchicine. The cells were then washed three times with medium alone and further incubated in the presence or absence of MCF for 72 hr. Medium was removed and assayed for PGE_2 concentration and collagenase activity. Values represent means ± S.E.M. of triplicate wells.

Effects of cytochalasin B, colchicine and trifluoperazine on MCF response. Observations that PGE_2 markedly stimulates cellular cAMP content and that PGE_2 is at least one factor responsible for the stellate shape of the synovial cells [8] led us to examine effects of drugs which influence the cytoskeleton. Some substances which act on components of the cytoskeleton (e.g. colchicine or cytochalasin B) have been shown to stimulate both collagenase and PGE_2 in several cell culture systems [17–19]. Several hormones which act at the cell membrane appear to affect at least two interdependent systems: levels of cAMP through adenylate cyclase and levels of intracellular calcium which may act through calmodulin. For these reasons, a drug which binds to Ca^{2+}-calmodulin complexes was also investigated. By utilizing such agents, the relationships between PGE_2 production and collagenase synthesis might be further defined. As shown in Table 2, preincubation for as short a period as 1 hr with either cytochalasin B (1 μM) or colchicine (0.1 μM) usually had a significant effect on the MCF stimulation. Similar results were also obtained with a preincubation period of 24 hr with both drugs (data not shown). The actions of these agents were more pronounced in augmenting the collagenase response to MCF as contrasted with that of PGE_2. A slight stimulation by the drugs themselves without MCF was also observed, even though these agents were not present during the subsequent 72 hr of incubation (Table 2). The potentiation was observed at the two different concentrations of either cytochalasin B or colchicine tested. Similar effects of these drugs alone on primary cultures were also observed although the range of stimulation was low

Table 3. Effects of trifluoperazine on MCF response in synovial cells*

Preincubation 1 hr	Incubation 72 hr MCF	Collagenase (units/10^6 cells/3 days) Expt. 1	Collagenase (units/10^6 cells/3 days) Expt. 2	PGE_2 (ng/10^6 cells/3 days) Expt. 1	PGE_2 (ng/10^6 cells/3 days) Expt. 2
Control	−	0.05 ± 0.04	0.02 ± 0.02	27 ± 5	13 ± 2
	+	4.55 ± 0.70	2.11 ± 0.79	628 ± 93	85 ± 16
Trifluoperazine, 50 μM	−	0.07 ± 0.03	0.01 ± 0.01	17 ± 2	5 ± 1
	+	14.59 ± 3.10	3.54 ± 1.28	171 ± 20	19 ± 1

* Two different strains of synovial cells at the second passage were preincubated for 1 hr with medium alone (control) or trifluoperazine (50 μM). The cells were then washed three times with medium alone and further incubated in presence or absence of MCF for 72 hr. Medium was removed and assayed for PGE_2 concentration and collagenase activity. Values represent means ± S.E.M. of triplicate wells.

Table 4. Comparative effects of indomethacin and trifluoperazine on stimulated (+MCF) and unstimulated (−MCF) synovial cells*

	Collagenase (units/10^6 cells/3 days) (−)MCF	Collagenase (units/10^6 cells/3 days) (+)MCF	PGE_2 (ng/10^6 cells/3 days) (−)MCF	PGE_2 (ng/10^6 cells/3 days) (+)MCF
Control	2.1	9.6	4.0	79.0
Indomethacin, 0.01 μM	3.7	9.8	0.1	0.5
Trifluoperazine, 25 μM	5.1	14.9	1.1	6.1

* Synovial cell strains at the second passage were incubated for 72 hr in the presence or absence of MCF with medium alone (control), indomethacin, or trifluoperazine. Medium was removed and assayed for PGE_2 concentration and collagenase activity. Values represent means ± S.E.M. of triplicate wells.

perhaps due to the high basal levels of collagenase and PGE_2 (data not shown).

We also observed that preincubation for as short a period as 1 hr with 50 μM trifluoperazine induced an augmentation of the MCF response by the synovial cells as measured by collagenase production but, in the same experiments, a decrease in MCF response as measured by PGE_2 production (Table 3). This dissociation of collagenase and PGE_2 response is similar to that observed in some experiments described earlier using low concentrations of indomethacin and raises the possibility that the collagenase increase is due solely to the decrease in PGE_2 levels. Therefore, experiments were designed to compare the effects of indomethacin and trifluoperazine on the same cells, as described in Table 4. Synovial cells were exposed to either drug with and without MCF stimulation, and collagenase and PGE_2 were measured after 3 days of culture under the conditions described. In cells without MCF, collagenase levels were increased slightly with low concentrations of indomethacin (10 nM) or trifluoperazine (25 μM). PGE_2 production was inhibited by ~40% at 1 nM indomethacin and by greater than 99% at 0.1 μM in the presence of MCF (data not shown). In contrast, when collagenase levels were stimulated above control by MCF in the presence of trifluoperazine, PGE_2 levels were clearly decreased. The results suggest that the collagenase stimulation by MCF in the presence of trifluoperazine is not PGE_2 dependent. The concentrations of trifluoperazine used did not alter cell viability, shape or number.

DISCUSSION

The rheumatoid synovial cell cultures, in which large amounts of latent collagenase and PGE_2 are synthesized and released into the medium either spontaneously or under MCF stimulation, provide a useful system for studying effects of drugs and for examining factors that control the degradative activity of the inflammatory tissue. In previous work from this laboratory and that of others, it has been shown that some agents (e.g. glucocorticoids) decrease production of both collagenase and PGE_2, whereas others (e.g. indomethacin) predominantly affect the levels of PGE_2 [3]. In the present report we have shown that collagenase production in early, active cultures was usually unaffected by addition of low concentrations (e.g. 1 nM) of indomethacin which, in contrast, blocked PGE_2 synthesis greater than ~50%. Even in the presence of MCF, such indomethacin concentrations usually did not inhibit and, in some instances actually increased medium collagenase levels. At higher concentrations of indomethacin (1–10 μM), which inhibited PGE_2 synthesis more than 95%, collagenase production was variably inhibited. This inhibition by indomethacin was reversed by the addition of small amounts of exogenous PGE_2. This suggests that collagenase inhibition is not a direct effect of the drug itself at this concentration but is mediated by PGE_2, although alterations in the concentration of other arachidonic acid metabolites might result from the action of indomethacin.

Depending upon the culture conditions, there is probably an optimal concentration of PGE_2 necessary for maximal collagenase production. The absolute level of PGE_2 is probably not the only determinant of the regulation of collagenase production; the relative sensitivity of the target cells to effects of PGE_2, such as increasing cellular cAMP content, is also important [11]. We suggest that exposure to indomethacin, which blocks PGE_2 release, will not only alter the basal cAMP content in the cells, but also their response to subsequent changes in ambient PGE_2 levels. The cAMP content may be one determinant of the cellular response to MCF. It should be emphasized, however, that MCF itself does not affect the adenylate cyclase system directly but does so via its effects on PGE_2 [11].

The findings in our experiments are consistent with those reported by Wahl *et al.* [17]. In murine peritoneal macrophages, addition of indomethacin to endotoxin- or muramyl dipeptide-stimulated cells resulted in a marked decrease in collagenase production [17, 18], suggesting a prostaglandin- and cAMP-dependent step in the synthesis of the enzyme. The addition of exogenous PGE_2 or dibutyryl cAMP restored collagenase production in the indomethacin-inhibited cultures [8, 18]. The addition of either exogenous PGE_2, or dibutyryl cAMP was not sufficient to initiate collagenase synthesis, however. In the synovial cells, addition of PGE_2 alone did not markedly affect basal collagenase production. Moreover, the collagenase dependence on prostaglandin synthesis in the murine macrophages is considerably greater than in the human synovial cells. Although PGE_2 could possibly function in the synovial cell system by inducing elevations in the intracellular concentration of cAMP, inhibition of MCF-induced PGE_2 synthesis by indomethacin results in no change in basal intracellular cAMP levels [11]. In the indomethacin-treated cells, which are exquisitely sensitive to PGE_2, a low ambient concentration of PGE_2 may be just sufficient to affect cell responses.

There are several lines of evidence in support of a functional role of cAMP in the regulation of microtubule assembly [15]. It was therefore of interest to evaluate the possible role of microtubules and microfilaments in the modulation of collagenase and PGE_2 production induced by MCF. It had also been shown in prior studies of organ and cell culture systems that agents such as colchicine or cytochalasin B can stimulate collagenase production [19–22].

We recently reported that the stellate morphology of the adherent synovial cells can be induced by PGE_2, presumably mediated by effects on adenylate cyclase, since addition of 8-bromo cAMP produces similar morphological changes [8]. MCF can also induce similar changes in late passage cells, which can be abolished by the addition of indomethacin but reproduced by the subsequent addition of PGE_2 to the cultures. Changes in cell shape are dependent upon the state of the cytoskeleton and are influenced by cAMP. However, the stellate configuration is *not* a requirement for collagenase production, since addition of indomethacin to MCF-treated cultures abrogates the morphological changes but has relatively little effect on collagenase. Furthermore, whereas preincubation with colchicine or cytochalasin B clearly potentiates the MCF stimulation and increases basal levels of collagenase, colchicine (but not cytochalasin B) blocks the development of the stellate appearance of the cells [8]. However, neither of these agents dissociates the MCF stimulation of collagenase and PGE_2 production. It is also unlikely that MCF acts solely as an endogenous colchicine-like factor, since MCF stimulation is over and above that produced by colchicine and the effects on cell shape, as discussed, are totally different.

Other control mechanisms implicated in microtubule assembly are related to levels of intracellular Ca^{2+} and Ca^{2+}-dependent protein phosphorylation [15, 23, 24]. Trifluoperazine is known to bind to the Ca^{2+}-calmodulin complex [25, 26] and acts to inhibit biological functions presumably carried out by this complex [27]. In contrast to the results obtained with colchicine and cytochalasin B, trifluoperazine while augmenting the stimulatory effects of MCF on collagenase synthesis blunts the stimulatory effects on PGE_2 synthesis. In our experiments preincubation or coincubation with trifluoperazine decreased PGE_2 synthesis but increased collagenase synthesis particularly in MCF-treated cultures. We cannot be certain that trifluoperazine binds to the Ca^{2+}-calmodulin in the cell since binding has been demonstrated only in cell extracts. However, the effects of trifluoperazine in augmenting the collagenase response to MCF are probably not all explainable by effects on PGE_2, since a similar decrease in PGE_2 levels produced by indomethacin does not augment MCF-stimulated collagenase synthesis. It is also possible that other actions of trifluoperazine, e.g. lipophilic effects on cell membranes, could contribute to this augmentation [28, 29]. For example, another phenthiazine derivative, chlorpromazine, has been shown to prevent the increased adenylate cyclase activity in bovine thyroid membranes induced by thyroid stimulating hormone (TSH) and PGE [30, 31]. Phenothiazines also inhibit phospholipase C in mouse macrophages [32]. Phosphatidic acid, a product of the reaction catalyzed by phospholipase C, could subsequently act as a calcium ionophore [33]. In other studies, it has been suggested that calmodulin can stimulate phospholipase A_2 directly [16]. By this mechanism, drugs which bind to Ca^{2+}-calmodulin complexes would therefore decrease PGE_2 production [34]. Calcium is also required for the stimulation of prostaglandin synthesis by tumor promotors such as certain phorbol esters [35]. Evidence has been obtained that phorbol ester receptors may be protein kinase C [36, 37], an enzyme which is activated by Ca^{2+}.

The major observations that have emerged from this study are that the ambient level of PGE_2 modulated collagenase production, probably mediated by effects of PGE_2 on adenylate cyclase and elevation of cellular cAMP content. Indomethacin affected collagenase production indirectly, by decreasing ambient PGE_2 levels, and the effects of the drug were reversed by restoring these PGE_2 levels. Trifluoperazine somehow dissociated collagenase from PGE_2 production under conditions where cells were stimulated by MCF. The mechanisms of these effects have not yet been elucidated. However, further studies which would provide insight into these

mechanisms would possibly lead to better strategies for therapy of the inflammatory process which leads to destruction of the extracellular matrix of the joint and other tissues.

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