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RAPID COMMUNICATION

INTERLEUKIN 1 ENHANCES SYNOVIAL CELL HYALURONATE SYNTHESIS

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Abstract. Interleukin 1 enhances proliferation of murine thymocytes in the presence of lectins, and is also known to stimulate the release of prostaglandins and neutral proteases from a variety of cell types. We have previously shown that a factor isolated from the culture media of disaggregated lining cells of the human synovial membrane was indistinguishable from monocyte-derived interleukin 1. We report here that interleukin 1 from either source stimulates hyaluronate synthesis by synovial membrane cells. Upon gel filtration or isoelectric focusing of synovial cell supernatants, the hyaluronate-stimulatory activity co-fractionates with the interleukin 1 activity. Enhanced cell secretion of hyaluronate is a newly described metabolic effect of interleukin 1.

Monocytes/macrophages activated by mitogens secrete factors into the supernatant media which have biological effects on a wide variety of cells. The factor that has received the most attention and been most completely characterized is interleukin 1 (IL 1), formerly known as lymphocyte activating factor (1). IL 1 is a 12,000 to 15,000 dalton polypeptide, and although its effects on B and T lymphocytes have been most intensively studied, IL 1 also has been shown to modulate the metabolism of mesenchymal cells, including synovial cells (2,3), human dermal fibroblasts (4), bone cells (5), and chondrocytes (6). Among the secretory products that IL 1 induces in mesenchymal cells are prostaglandins (PGE_2), neutral proteases and collagenase (3,6).

We have previously reported (7) that human synovial cells secrete a factor that by a variety of biochemical and immunological parameters appeared identical to macrophage-derived IL 1, and to which we refer in this paper as synoviocyte-derived IL 1. Since the synthesis of glycosaminoglycans (GAG) is one of the principal metabolic functions of synovial cells (8), it was of interest to determine the effects of macrophage-derived or synoviocyte-derived IL 1 on GAG production by synovial cells. The finding of augmented hyaluronate production induced by IL 1 adds a new metabolic effect to those already demonstrated for IL 1.

Materials and Methods. Collagenase, trypsin, and testicular hyaluronidase were from Sigma Chemical Co., St. Louis, MO; streptomyces hyaluronidase and chondroitinase ABC were from Miles Laboratories, Elkert, IN. $Na_2\ ^{35}SO_4$ (232.7mCi/mmol, (^{14}C) glucosamine hydrochloride (325.5mCi/mmol), Omnifluor™ and Riafluor™ were from New England Nuclear, Boston, MA. Newborn calf serum and Dulbecco's modified Eagle's medium were from Grand Island Biological, Grand Island, NY. Thin layer (30x20 cm) cellulose-coated plastic sheets without fluorescent indicator were from Merck, Darmstadt, West Germany. Sephadex G 75 was from Pharmacia, Piscataway, NJ.

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Synovial Tissue. Surgical specimens of synovial membranes obtained from total joint replacement were transported to the laboratory in chilled Dulbecco's modified Eagle's medium without serum. The synovial cells were disaggregated and cultured as previously described (8). Briefly, the tissue was cut into pieces of 2mm or less and incubated with 0.1 mg/ml testicular hyaluronidase and 1 mg/ml collagenase at 37°C with rocking. After 4 hours, the suspension was centrifuged at 1000 rpm for 10 minutes, and the supernatant saved. This was the starting material for the synoviocyte-derived factor characterized as interleukin 1 (7). The synovial cells were resuspended in medium with 10% newborn calf serum for cell culture at 37°C in 5% CO_2.

Synovial Cell Cultures. GAG and PGE_2 synthesis: When the adherent synovial cells become confluent (approximately $5x10^5$ cells/35mm dish), the medium was changed and 2 ml of fresh medium containing the IL 1 sample to be tested was added. Plates with medium alone served as controls. After 24 hours incubation, a 200 ul sample of medium was removed for PGE_2 analysis (9), and isotopic precursors of GAG were added: 1.5uCi ^{14}C glucosamine, or 30 uCi (^{35}S) sulfate in 2 ml medium. After 24 hours further incubation, medium was removed and analyzed for labeled GAG according to methods described elsewhere (8). Hyaluronate was determined by the ^{14}C radioactive counts rendered dialyzable by streptomyces hyaluronidase digestion compared to undigested samples. Total GAG containing ^{14}C or ^{35}S were determined by radioactive counts rendered dialyzable by chondroitinase ABC compared to undigested samples.

Chromatography of GAG. GAG were isolated, digested with chondroitinase ABC, and subjected to thin layer chromatography and radioautography by methods described in detail elsewhere (8). The individual radioactive spots on the cellulose-coated plastic sheet were cut out, placed in Omnifluor™ and cpm were determined in a scintillation counter. The piece of cellulose-coated plastic was then washed with toluene to remove the fluor, air-dried, and the cellulose scraped into a test tube for analysis of acetylhexosamine (10).

Preparation of Monocyte-derived IL 1. The monocyte-derived IL 1 was prepared as previously reported (11) from buffy coat preparations of human peripheral blood.

Purification of IL 1. Purification of IL 1 derived from the disaggregated synovial cell supernatant was carried out first by affinity chromatography on columns of Sepharose-bound rabbit anti-human leukocyte pyrogen (12) and then gel filtration on columns of Sephadex G-75. Monocyte-derived IL 1 was purified initially on Sephadex G-75. Some samples were further purified by isoelectric focusing (11).

Assays for IL 1. IL 1 was assayed by the thymocyte comitogenic test (11), and was further characterized by induction of interleukin 2 (13) and of serum amyloid A protein (11). One unit of IL 1 is defined as the amount necessary to induce a half maximal response in the thymocyte proliferative assay.

Results and discussion. Several experiments with macrophage-derived, and one experiment with synoviocyte-derived IL 1 on GAG synthesis by synovial cells are shown in Table 1. These IL 1 preparations generally increased ^{14}C-glucosamine incorporation into GAG. The augmented GAG production was due largely to increased hyaluronate synthesis since most of the ^{14}C label was digested by streptomyces hyaluronidase, an enzyme specific for hyaluronate (11). The T test of differences for both total GAG and hyaluronate results were significant, each having a value of <0.05.

^{35}S incorporation into GAG was generally not increased by IL 1. The stimulation of sulfated GAG synthesis occasionally obtained with IL 1 may be due in part to variable synthesis of the sulfated GAG themselves (8) which are more strongly influenced by culture conditions and the cell type that evolves in culture (14).

Table 1 also shows that IL 1 augmented PGE_2 synthesis by synovial cells.

TABLE 1. EFFECTS OF IL 1 ON GLYCOSAMINOGLYCAN SYNTHESIS AND PGE_2 PRODUCTION BY SYNOVIAL CELLS

		cpm/10^5 cells in: (stimulation index)			
		Total Glycosaminoglycans	Hyaluronate	Sulfated Glycosaminoglycans	PGE_2
Exp.	Conditions[a]	(cpm ^{14}C)	(cpm ^{14}C)	(cpm ^{35}S)	(pG/ml)
1	Control	8097	7254	2146	
	IL 1	9976 (1.2)	9392 (1.3)	8132 (3.8)	
2	Control	246	244	1805	
	IL 1	3561 (14.5)	3294 (22.9)	1306 (0.7)	
3	Control	4384	3519	59,660	$< 5 \times 10^2$
	IL 1	10,066 (2.3)	9285 (2.6)	27,889 (0.5)	1.4×10^3
4	Control	41,862	11,591		$< 5 \times 10^2$
	IL 1	57,897 (1.4)	12,005 (1.03)		2.3×10^4
	IL 1 + indo.	52,902 (1.3)	13,510 (1.16)		$< 5 \times 10^2$
	Indo.	35,200 (0.8)	12,137 (1.05)		$< 5 \times 10^2$
5	Control	2888	2257		$< 5 \times 10^2$
	IL 1	12,937 (4.5)	13,260 (5.9)		4.1×10^3
6	Control	265	232		3.4×10^3
	IL 1	1021 (3.9)	969 (4.2)		5.0×10^4

a Experiments 1-5, monocyte derived IL 1 (15,000 dalton, pI 7.1) 100 units per plate; Experiment 6, synoviocyte derived IL 1 10 units/plate. Indomethacin (indo) was used at 1.5×10^{-5}M.

Evidence has been presented that PGE_2 itself is able to enhance hyaluronate synthesis (15), but in our experiments the increased hyaluronate production by IL 1 could not be attributed to a rise in PGE_2 levels in the cell cultures. Table 1 shows that indomethacin prevented the IL 1-induced rise of PGE_2 without greatly affecting hyaluronate synthesis.

Thin layer chromatography confirmed that hyaluronate was the predominant ^{14}C labeled GAG isolated from the medium of synovial cell cultures (Fig.1). Table 2 also shows that both synoviocyte and macrophage derived IL 1 enhanced the incorporation of ^{14}C glucosamine into hyaluronate. The total amount of N-acetylhexosamine does not increase as dramatically as does the counts because of the pool of unlabeled hyaluronate. Under the same conditions, no increase was observed in the incorporation of ^{35}S into GAG.

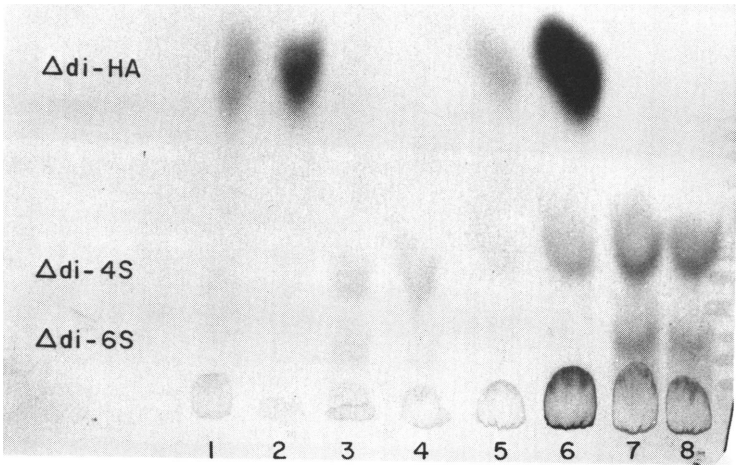

FIG. 1. Thin layer chromatogram developed by radioautography of the labeled GAG isolated from the media of synovial cells and digested with chondroitinase ABC (8). Location of authentic unsaturated disaccharide standards are shown: Δdi-HA from hyaluronate (HA); Δdi-4S from chondroitin 4-sulfate; Δdi-6S from chondroitin 6-sulfate. 1 and 3 = control synovial cells (^{14}C and ^{35}S, respectively), 2 and 4 = effects on synovial cells of synoviocyte-derived IL 1 (^{14}C and ^{35}S); 5 and 7 = control synovial cells (^{14}C and ^{35}S); 6 and 8 = effects on synovial cells of macrophage derived IL 1 (^{14}C and ^{35}S).

TABLE II. RADIOACTIVE COUNTS AND N-ACETYLHEXOSAMINE CONTENT IN THE UNSATURATED DISACCHARIDES SEPARATED BY THIN LAYER CHROMATOGRAPHY (SEE FIG. 1)

Conditions	Unsaturated Disaccharide	cpm ($^{14}C/10^5$ cells)	N-acetylhexosamine (ug/10^5 cells)	cpm ($^{35}S/10^5$ cells)
Control	HA	1489	2.0	
	CS-4			442
	CS-6			267
Synoviocyte IL 1	HA	3848	2.5	
	CS-4			457
	CS-6			186
Control	HA	1106	1.25	
	CS-4	298		1936
	CS-6	179		1506
Macrophage IL 1	HA	15,723	3.5	
	CS-4	1687		1420
	CS-6	417		784

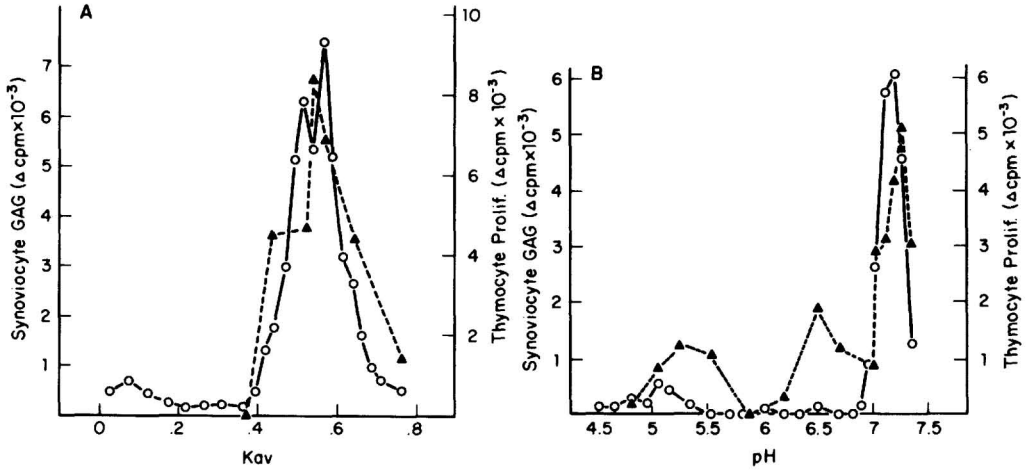

FIG. 2. Cofractionation of synoviocyte-derived IL 1 and the factor that stimulates GAG synthesis. The synoviocyte-derived IL 1 was partially purified by affinity chromatography over Sepharose-bound rabbit anti-human leukocytic pyrogen. (A) The IL 1 was further fractionated by chromatography over Sephadex G75. Fractions were assayed either for their ability to enhance GAG synthesis by adherent human synoviocytes (Δ) or to enhance the proliferation of C3H/HeJ thymocytes with phytohemagglutinin (O). (B) The fractions eluting with a Kav of 4.5-6.5 were pooled and further analyzed by isoelectric focusing.

To determine whether IL 1 is indeed the factor responsible for enhanced GAG synthesis, the biochemical properties in chromatographically purified synoviocyte derived IL 1 is shown in Fig.2. The fractions with the highest thymocyte comitogenic activity also showed the ability to augment synovial cell hyaluronate synthesis whether they were obtained by gel filtration or by isoelectric focusing. These fractions also enhanced PGE_2 production by synovial cells (7).

In contrast to the use of well characterized IL 1 reported here, less clearly defined mononuclear cell culture media supernatants have been used by others to study GAG synthesis by mesenchymal cells. The predominant effect reported has been to augment synovial cell hyaluronate synthesis (16,17). A connective tissue activating peptide (CTAP) derived from platelets (I) and lymphocytes (III) also stimulated GAG synthesis by synovial cells (15). CTAP-I differs from IL 1 in its more strongly cationic nature and its inability to stimulate synthesis by synovial cells in the presence of a prostaglandin antagonist (15).

In conclusion, cellular events mediated by synoviocyte-derived IL 1 could play a role in mesenchymal cell response to arthritic diseases by promoting degradation of connective tissue components through the release of collagenase, proteases, and PGE_2, or repair by virtue of inducing hyaluronate synthesis.

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