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Stimulation of rheumatoid synovial cell collagenase and prostaglandin production by partially purified lymphocyte-activating factor (interleukin 1)

(macrophage/rheumatoid arthritis)

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ABSTRACT **Human macrophages produce in culture a factor termed mononuclear cell factor (MCF) that increases the production of collagenase and prostaglandins by isolated adherent rheumatoid synovial cells. A factor with similar biologic activity is also produced by the murine macrophage cell line P388D$_1$. By using a sequential purification scheme involving ammonium sulfate fractionation; chromatography on DEAE-cellulose, Sephacryl S-200, and phenyl-Sepharose; and discontinuous polyacrylamide gel electrophoresis, the P388D$_1$ cell-derived, synovial cell-stimulating factor was copurified with the lymphocyte-activating factor [LAF; interleukin 1 (IL 1)]. The specific activity of the partially purified LAF (IL 1) was approximately 15,000-fold higher than that of the LAF (IL 1) in the original P388D$_1$ cell culture supernatant. On the basis of (*i*) the copurification of the P388D$_1$ cell-derived LAF (IL 1) and synovial cell-stimulating factors; (*ii*) the similarity in cell of origin, molecular weight, and phenylglyoxal sensitivity of human MCF and murine LAF (IL 1); and (*iii*) the presence of LAF (IL 1) activity in preparations of partially purified human MCF, we have postulated that LAF (IL 1) may have effects on cell targets that are nonlymphoid in nature and also that human MCF may be similar to, or identical with human LAF (IL 1). The results of these studies have raised the possibility that LAF (IL 1) may play a role in macrophage-mediated activation of synovial cells and lymphocytes which are involved in the inflammatory responses associated with rheumatoid arthritis.**

Isolated human adherent synovial cells obtained from rheumatoid synovectomy preparations produce in primary culture large quantities of collagenase and prostaglandins (1). Although the precise cellular nature of the adherent synovial cells is not established, these cells do not possess the morphological or functional markers of macrophages or lymphocytes and are different from normal dermal fibroblasts in that they produce less collagen per cell. The ability of adherent synovial cells *in vitro* to produce collagenase and prostaglandins declines with time in culture but can be restored by addition of supernatant media from cultures of human peripheral blood mononuclear cells (2, 3). The active factor in the human mononuclear cell culture supernatant (mononuclear cell factor, MCF) has a molecular weight of 10,000–20,000 (2, 3) and has been shown to be macrophage-derived (4). The molecular weight of MCF is similar to that of another factor produced by macrophages, the lymphocyte-activating factor [LAF; interleukin 1 (IL 1)]‡ (5, 6). LAF (IL 1) is genetically unrestricted, an antigen-nonspecific, and species-unrestricted peptide that stimulates a variety of T cell-dependent processes—e.g., thymocyte proliferation (5, 6), *in vitro* antibody responses (7), and alloantigen-specific, T cell-mediated cytotoxicity (8). In addition, LAF (IL 1) may be similar to the macrophage-derived endogenous pyrogen (9, 10). In view of the similarities with respect to cell of origin and molecular weight between MCF and LAF (IL 1), we initiated studies to determine if MCF and LAF (IL 1) were functionally and chemically related. The results of our studies suggest that (*i*) LAF (IL 1) may possess the ability to stimulate collagenase and prostaglandin production by human rheumatoid synovial cells and (*ii*) human MCF may be similar to or identical with human LAF (IL 1).

MATERIALS AND METHODS

Synovial Cell Culture. Isolated adherent rheumatoid synovial cells were prepared from rheumatoid arthritis synovectomy specimens by sequential treatment with proteolytic enzymes (1–4, 11). Cultures of synovial cells were prepared and maintained as described (1–4). For these studies, adherent synovial cells were plated at the second passage in 16-mm-diameter wells (Costar, Cambridge, MA) at 1×10^5 cells per well. At the second passage, the basal rates of collagenase and prostaglandin E_2 production by synovial cells were usually lower than in the primary cultures, which permitted measurement of a dose-related increase in the production of these two substances. For assay of the MCF activity in various fractions, the adherent synovial cells were incubated in RPMI 1640 medium containing 1% (vol/vol) fetal calf serum for 3 days. Cell-free culture fluid was then prepared and assayed for collagenase and prostaglandin E_2 activities.

Production and Partial Purification of MCF. MCF was prepared from unstimulated cultures of human peripheral blood mononuclear cells as described (11). The MCF in the culture fluid was partially purified by gel filtration on columns of Ultrogel AcA54 as described (3). In some cases, the samples were first chromatographed on hydroxyapatite columns as follows. Supernatant culture media were dialyzed at 4°C against 2 mM sodium phosphate (pH 7.2) and then applied onto columns containing 10 ml of hydroxyapatite (Bio-Gel HTP, Bio-Rad) equilibrated with the same buffer. Fractions containing MCF activity were eluted with 2.5 column volumes of 180 mM sodium phosphate (pH 7.2).

Production and Purification of LAF (IL 1). LAF (IL 1) was prepared from culture fluids of phorbol 12-myristate 13-acetate-

Abbreviations: MCF, mononuclear cell factor; LAF (IL 1), lymphocyte activating factor (interleukin 1).

‡ A revised nomenclature for lymphocyte-activating factor (LAF) was proposed at the Second International Lymphokine Workshop (Ermatingen, Switzerland, May 27–31, 1979). The revised term for LAF is interleukin 1. To avoid confusion, the term IL 1 will be initially assimilated into the literature by using both acronyms as follows: LAF (IL 1). In time the term LAF will be deleted.

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Table 1. Correlation between LAF (IL 1) and MCF activities in partially purified material obtained from P388D$_1$ cells and human mononuclear cells

Sample	LAF (IL 1) activity,* units/ml	MCF activity,† units/10^6 cells
Experiment 1‡		
LAF (IL 1)	50	3.57
	25	3.48
	12.5	2.98
	6.25	2.64
	3.12	1.29
	1.56	1.02
	0.78	0.48
	0	0.08
Experiment 2§		
MCF (Ultrogel AcA54)	2.3	37.14
	1.15	5.79
	0.58	2.14
	0	0.10
MCF (hydroxyapatite + Ultrogel AcA54)	0.094	0.45
	0.047	0.21
	0.024	0.11
	0	0.10

* The units of LAF (IL 1) activity were determined by using an internal LAF (IL 1) standard (11).

† The MCF activity refers to the collagenase activity of synovial cells incubated with dilutions of the LAF (IL 1) preparation. 1 unit of collagenase activity equals 1 μg of collagen fibrils degraded per min at 37°C.

‡ C3H/HeJ mouse thymocyte cells [LAF (IL 1) activity] or human adherent synovial cells [MCF activity] were incubated with varying concentrations of P388D$_1$ cell-derived LAF (IL 1).

§ Thymocyte or human adherent synovial cells were incubated with various concentrations of human mononuclear cell-derived MCF that has been fractionated on Ultrogel AcA54 or hydroxyapaptite and Ultrogel AcA54. It should be noted that separate synovial cell preparations were used in experiments 1 and 2.

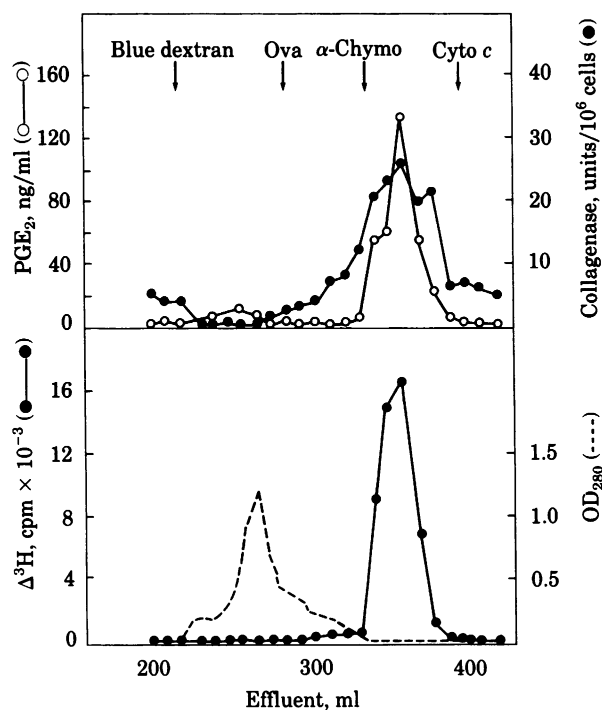

FIG. 1. Sephacryl S-200 chromatography of concentrated culture media from P388D$_1$ cells. Approximately 200 ml of culture media was concentrated to 5 ml and applied to the column. (*Upper*) Synovial cell production of prostaglandin E_2 (PGE_2) (○——○) and collagenase (●——●). Fractions were diluted 50% and tested for MCF activity. (*Lower*) LAF (IL 1) activity (●——●) measured by C3H/HeJ thymocyte cell proliferation [Δ cpm of ^{3}H-TdR incorporation ($\times 10^{-3}$)]. Fractions were diluted 25% and tested for activity. Ova, ovalbumin; α-Chymo, α-chymotrypsinogen; Cyto c, cytochrome c.

stimulated P388D$_1$ cells (12). For some experiments, the crude supernatant media were concentrated by using an Amicon UM10 ultrafiltration membrane prior to chromatography on a 90 × 2.7 cm Sephacryl S-200 (superfine) gel filtration column (12). LAF (IL 1) was purified by a sequence of steps, including ammonium sulfate fractionation, DEAE-cellulose batch elution, Sephacryl S-200 chromatography, and phenyl-Sepharose hydrophobic chromatography (12, 13). The resultant material, which had a specific activity of approximately 20,000 units/mg of protein (activity of the crude supernatant = 18 units/mg of protein), was then electrophoresed on 10% Tris glycinate discontinuous polyacrylamide gels (13). The peak fractions of gel-purified LAF (IL 1) had a specific activity of approximately 3 × 10^5 units/mg of protein.

Bioassays. Prostaglandins in the culture fluid of adherent synovial cells were measured by radioimmunoassay with an antiserum to prostaglandin E_2 (1–3) in the laboratory of D. R. Robinson, Massachusetts General Hospital. Collagenase activity was assayed by solubilization of reconstituted [^{14}C]glycine-labeled guinea pig skin collagen fibrils as described (1–3). Protein was determined by the method of Lowry *et al.* (14) or Spector (15) with collagen or bovine serum albumin as standards. C3H/HeJ mouse thymocyte proliferation [LAF (IL 1) activity] was measured as described (12, 13). The units of LAF (IL 1) activity were determined by dilution analysis of test samples and a standard preparation of partially purified LAF (IL 1) (100 units/ml) (13). Fifty percent of the maximal thymocyte proliferation response is generally obtained with 5–10 units of LAF (IL 1) per ml.

RESULTS

Because the murine macrophage cell line P388D$_1$ produces a LAF (IL 1) that is indistinguishable from that produced by normal murine macrophages (6), we used P388D$_1$ cells as a source of material to evaluate the possible relationship between MCF and LAF (IL 1). Stimulation of P388D$_1$ cells with phorbol 12-myristate 13-acetate resulted in the production and secretion of both LAF (IL 1) and MCF activities (Table 1). When concentrated culture media from phorbol 12-myristate 13-acetate-stimulated P388D$_1$ cells were chromatographed on a Sephacryl S-200 column, LAF (IL 1) and the synovial cell-stimulating factor were coeluted in the molecular weight range of 10,000–25,000 (Fig. 1). The peak activity of both factors was eluted with an apparent molecular weight of approximately 16,000. Thus, the P388D$_1$ cells produced a MCF with the same apparent molecular weight as those of LAF (IL 1) and the human MCF (3).

Preparations highly enriched for LAF (IL 1) can be obtained from the culture fluid of P388D$_1$ cells by using a number of conventional protein-purification procedures. After phenyl-Sepharose chromatography, the specific activity of the LAF (IL 1) was increased approximately 1200-fold. When this material was further purified by electrophoresis on 10% (wt/vol) tris glycinate discontinuous polyacrylamide gels, LAF (IL 1) was consistently detected as two closely migrating species with specific activities of >3 × 10^5 units/mg of protein (13) (Fig. 2). The peaks of the synovial cell collagenase- and prostaglandin-stimulating activity were found in the same fractions that contained

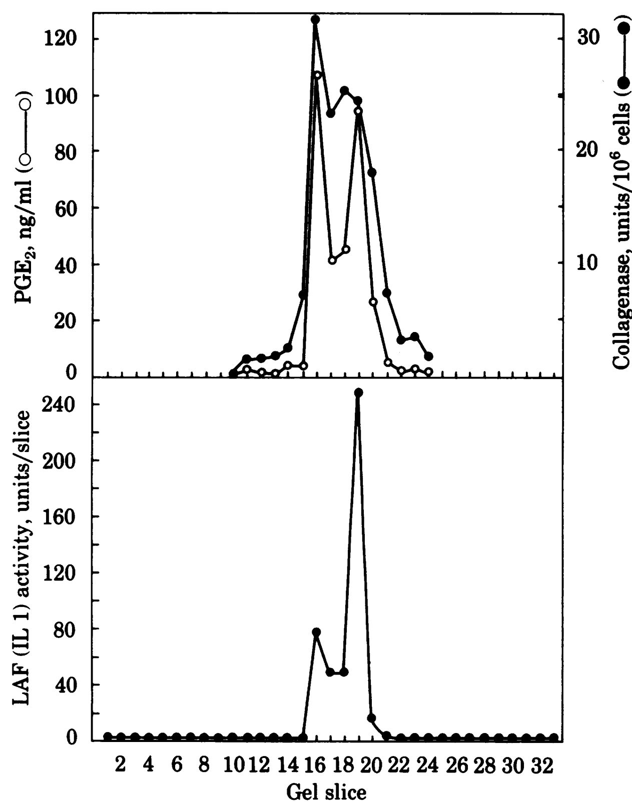

FIG. 2. Tris glycinate/polyacrylamide gel electrophoresis of purified P338D$_1$ cell-derived LAF (IL 1). Highly purified LAF (IL 1) was electrophoresed on 10% gels. (*Upper*) The gel slices (2 mm) were tested at a 50% dilution for MCF activity [synovial cell production of prostaglandin E$_2$ (PGE$_2$) and collagenase]. (*Lower*) The units of LAF (IL 1) activity in each gel slice were determined by the thymocyte proliferation response test (12, 13).

LAF (IL 1). However, the basic component of LAF (IL 1) was apparently more active in terms of MCF activity than the acidic component of LAF (IL 1) was. Nonetheless, these results demonstrate that the P388D$_1$ cell-derived LAF (IL 1) and synovial cell-stimulating factor are copurified through five distinct biochemical fractionation procedures.

In a previous study on the structure–function relationships of LAF (IL 1), we found that the specific modification of arginine groups in LAF (IL 1) by phenylglyoxal (16) resulted in a loss of biologic activity in the thymocyte proliferation assay (13). When a partially purified preparation of P388D$_1$ cell-derived LAF (IL 1) was treated with phenylglyoxal and tested for its ability to stimulate synovial cell collagenase production and thymocyte proliferation, we observed that both activities were markedly reduced (Table 2).

Murine LAF (IL 1) is not irreversibly denatured by 8 M urea and is only weakly sensitive to papain when the LAF (IL 1) is in its native configuration. However, in the presence of 8 M urea at 37°C, LAF (IL 1) is destroyed by papain (12). Therefore, we investigated whether the MCF activity of partially purified LAF (IL 1) was (*i*) resistant to irreversible denaturation by urea and (*ii*) susceptible to papain attack in 8 M urea. As shown in Table 3, the time course for the loss of LAF (IL 1) and MCF activities was identical in the presence of papain in 8 M urea. Both activities were unaffected by papain during the first 10–30 min of incubation but were markedly diminished by 60 min and were completely lost after 240 min. It also should be emphasized that both activities were not irreversibly affected by the urea.

The results of these physiochemical studies with P388D$_1$ cell-derived material support the hypothesis that LAF (IL 1) possesses the ability to stimulate not only lymphocyte activation but also collagenase and prostaglandin production by the nonlymphoid rheumatoid synovial cells. In addition, these results raise the possibility that human MCF may be identical to human LAF (IL 1).

In support of this hypothesis we also found that partially purified human MCF possesses thymocyte proliferation activity (Table 1). As is the case with P388D$_1$ cell-derived LAF (IL 1), the ability of MCF to stimulate thymocyte proliferation was proportional to its ability to augment synovial cell collagenase and prostaglandin production. Furthermore, when human MCF was treated with phenylglyoxal to modify arginine residues, its ability (like that of P388D$_1$ cell-derived LAF (IL 1)) to stimulate synovial cells was markedly diminished (Table 2).

DISCUSSION

The results presented in this communication demonstrate that preparations highly enriched for murine macrophage cell line LAF (IL 1) stimulate not only the proliferation and functional maturation of T lymphocytes (5–8, 12, 13) but also the production of collagenase and prostaglandins by the nonlymphoid rheumatoid synovial cells. Our results also support the hypothesis that MCF activity may be a property of human LAF (IL 1). Because the human material used in these studies was not highly purified but only partially purified by using gel filtration and hydroxyapatite chromatography, it is quite possible that other factors besides LAF (IL 1) may have contributed to the overall MCF activity of a given human preparation. Therefore, the definitive proof that human LAF (IL 1) is singularly responsible for the MCF activity in our human monocyte-derived

Table 2. Effect of phenylglyoxal on LAF (IL 1) and MCF activities

Preparation	Phenylglyoxal*	Residual LAF (IL 1) activity,† units/ml	Collagenase production in sample dilutions,‡ units/10^6 cells		
			1/4	1/8	1/16
P388D$_1$-LAF (IL 1)	−	25	4.31 ± 0.62	1.61 ± 0.41	0.36 ± 0.1
	+	6.25	2.76 ± 0.18	0.31 ± 0.1	0.26 ± 0.05
Human MCF	−	N.D.§	7.2 ± 0.08	3.83 ± 0.25	2.14 ± 0.18
	+	N.D.§	3.97 ± 0.38	0.71 ± 0.03	0.18 ± 0.01

* P388D$_1$ cell-derived LAF (IL 1) or human MCF were incubated for 4 hr at 25°C with 1 mM phenylglyoxal in 200 mM imidazole buffer (pH 8.0). The LAF (IL 1) and MCF were separated from unreacted phenylglyoxal by filtration on columns of Sephadex G-25 (12).

† Following incubation in the presence or absence of phenylglyoxal.

‡ MCF activity as measured by stimulation of synovial cell collagenase production.

§ N.D., not determined.

Table 3. Papain sensitivity of $P388D_1$ cell-derived LAF (IL 1) and MCF activities

Activity	Papain exposure time,* min	Activity, % remaining
Murine thymocyte proliferation [LAF (IL 1)]	0	100
	10	84.6
	60	1.3
	240	0
Synovial cell prostaglandin production (MCF)	0	100
	10	84.6
	60	38.5
	240	0

* $P388D_1$ cell-derived LAF (IL 1) (8 μg/ml; specific activity, 38,500 units/mg of protein) was incubated for various periods with 100 μg of papain per ml of 8 M urea/0.5 mM EDTA/50 μM 2-mercaptoethanol/3.7 mM cysteine/20 mM sodium phosphate, pH 6.2. The papain was activated for 30 min at 25°C prior to addition of the LAF (IL 1). At each time point, the sample was passed over a 0.2-ml agarose-Gly-Gly-Tyr(Bzl)-Arg column (Miles-Yeda) to remove the papain. The samples were then dialyzed against phosphate-buffered saline and tested for LAF (IL 1) and MCF activities. The initial LAF (IL 1) activity of the sample was 32 units/ml, and the starting material also induced 13 ng of prostaglandin E_2 per 10^5 human synovial cells.

preparation must await the availability of highly purified human LAF (IL 1). Nonetheless, our findings are certainly consistent with the view that human LAF (IL 1), like the murine product, may possess potent synovial cell-activating activity.

Recently, Newsome and Gross (17) reported that collagenase production by stromal cells from injured rabbit cornea could be markedly enhanced by a factor obtained from rabbit mononuclear cells. Similarly, Deshmukh-Phadke and coworkers (18, 19) found that a 12,000–14,000 molecular weight rabbit peritoneal macrophage product stimulated chondrocyte collagenase and neutral protease production. In view of our results, it is quite possible that these factors are also related to LAF (IL 1). Although some of the murine LAF (IL 1) preparations used here were purified more than 15,000-fold and still had synovial cell-stimulating activity, the possibility remains that the two biological activities are associated with different, but biochemically similar molecules. However, the concept that LAF (IL 1) may have a spectrum of biologic activities that extends beyond lymphocyte targets is supported by the recent finding that LAF (IL 1) may be similar to or identical with the macrophage-derived endogenous pyrogen (9, 10). Whereas human and murine LAF (IL 1) exhibit the same molecular weight and spectrum of biological activities (5–7), they differ in isoelectric point and relative sensitivity to proteolytic attack (5). Human LAF (IL 1) possesses pI values between 6.5 and 7.0, whereas mouse LAF (IL 1) exhibits pI values between 4.8 and 5.7. Interestingly, human and mouse LAF (IL 1) exhibit a similar pattern of heterogeneity on polyacrylamide gels (Fig. 2; ref. 7) and DEAE-cellulose (6, 7). Thus, although biologically and biochemically related, human LAF (IL 1) (MCF) and $P388D_1$-derived LAF (IL 1) most likely have significant differences in their primary amino acid sequence.

The pathologic events in the joints of patients with rheumatoid arthritis are associated with the infiltration of the synovial layers by various classes of cells, including macrophages and lymphocytes (20). Analysis of the T lymphocytes in synovial fluid from patients with rheumatoid arthritis indicates that these cells are activated in terms of proliferation, *stable* E-rosette-forming capacity, and macrophage-activating activity (21). In view of the ability of LAF (IL 1) to stimulate T-cell proliferation and the generation of *stable* E-rosette-forming T cells (22), it is possible that macrophages in the synovium through their elaboration of a single factor LAF (IL 1) may regulate the inflammatory activity of both T lymphocytes and synovial cells.

Note Added in Proof. $P388D_1$-derived LAF (IL 1) has been purified to homogeneity (23). The purified LAF (IL 1) was highly active in the induction of synovial cell collagenase synthesis.

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