Participation of Monocyte-Macrophages and Lymphocytes in the Production of a Factor that Stimulates Collagenase and Prostaglandin Release by Rheumatoid Synovial Cells

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ABSTRACT Cultured mononuclear cells from human peripheral blood produce a soluble factor (MCF) that stimulates collagenase and prostaglandin E_2 (PGE_2) release by cultured rheumatoid synovial cells up to several hundredfold. These target rheumatoid synovial cells lack conventional macrophage markers. To determine which mononuclear cells are the source of MCF, purified populations of monocyte-macrophages, thymus-derived (T) lymphocytes, and bone-marrow-derived (B) lymphocytes were prepared. The monocyte-macrophages alone produced levels of MCF that were proportional to cell density but unaffected by phytohemagglutinin or pokeweed mitogen. No detectable collagenase activity was produced by the cultured monocyte-macrophages or lymphocytes. Purified T lymphocytes produced levels of MCF ≅1–3% those of purified monocyte-macrophages in the presence or absence of the above lectins. Purified T lymphocytes modulated the production of MCF by the monocyte-macrophages, however, in a manner dependent upon relative cell densities and the presence of lectins. For example, at optimal ratios of T lymphocytes: monocyte-macrophages, MCF production was markedly stimulated by pokeweed mitogen. Thus, interactions of T lymphocytes and monocyte-macrophages could be important in determining levels of MCF, which regulate collagenase and PGE_2 production by target synovial cells in inflammatory arthritis.

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INTRODUCTION

The cell-cell interactions and mechanisms important in the joint destruction observed in patients with chronic rheumatoid arthritis have not been precisely identified (1–4). It is known that the pannus includes synovial lining "A" and "B" cells, lymphocytes, plasma cells, phagocytic cells, uncharacterized multinucleated giant cells, fibroblasts, and the endothelial cells of the blood vessel walls, among others (5–10). Interactions among these cells are likely to be important in determining the character, tempo, and intensity of the destructive process. We have isolated adherent rheumatoid synovial cells (ASC)[1] from the superficial layer of synovectomy specimens. These cells lack conventional macrophage markers, but early in culture produce and continue to release high levels of collagenase and prostaglandin E_2 (PGE_2) (11). These two products may contribute significantly to the destructive processes that characterize rheumatoid arthritis (4, 12). In previous studies, we have shown that cultured mononuclear cells, including lymphocytes and monocytes from normal human peripheral blood, produce a soluble factor (≅14,000 daltons) that stimulates the ASC to produce more collagenase (13) and prostaglandin (14). These observations taken together suggest that one or more soluble factors released from mononuclear cells in the inflammatory synovium in rheumatoid arthritis may play a biologic role in subsequent joint destruction.

[1] *Abbreviations used in this paper*: ASC, adherent rheumatoid synovial cells; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; Ig^-, nonimmunoglobulin-bearing; Ig+, immunoglobulin-bearing; PGE_2, prostaglandin E_2; PHA, phytohemmagglutinin; PWM, pokeweed mitogen.

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In the present studies, we used several cell separation techniques to isolate purified populations of T cells, B cells, and monocytes from peripheral blood to determine the predominant cell type responsible for the production and release of the collagenase- and PGE_2-stimulating factor. Experiments were designed to determine if interactions among these purified populations are important in the control of production of the collagenase- and PGE_2-stimulating factor. We will present evidence that highly purified adherent monocytic populations alone in the absence of mitogens secrete the stimulating factor. Further, we will show that purified T lymphocytes alone do not secrete the factor, but when cocultivated with monocytes and upon activation by mitogens they may modulate factor production by monocytes. The role of B lymphocytes in production of the stimulating factor is more difficult to determine, since that produced by highly purified populations of B lymphocytes could be accounted for by residual (<5–10%) contaminating monocytes.

METHODS

Target cells: human rheumatoid synovial cells. All cells used for bioassay were obtained from surgical synovectomy specimens from patients with rheumatoid arthritis. Isolated ASC were prepared by proteolytic dispersion as described (3). Cells were carried in 100-mm diameter polystyrene tissue culture dishes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) with Dulbecco's modified Eagle's medium (DMEM) (Grand Island Biological Co., Grand Island, N. Y.) containing 10% fetal calf serum (FCS; Microbiological Associates, Walkersville, Md.), and 100 U/ml penicillin and 100 μg/ml streptomycin (Grand Island Biological Co.). Cultures were incubated at 37°C in an atmosphere of 95% air and 5% CO_2. Passages were made after treatment with trypsin-EDTA (Grand Island Biological Co.) at dilutions 1:2 or 1:4. For bioassays, ASC were plated 3–6 d earlier in culture wells 16 mm in diameter (Costar, Data Packaging, Cambridge, Mass.) at densities of $5–10 \times 10^4$ cells/well with 0.5 ml medium/well. Different preparations of ASC target cells were used in each of the experiments described below. Sufficient numbers of cells could not be obtained or carried from any one synovectomy specimen to perform all of the studies to be described. Therefore it was necessary to compare responses in different cells that did not have the same sensitivity to a single preparation of mononuclear cell culture medium. Results within any biological assay were interpreted with respect to that target cell preparation.

Collagenase assay. For assays of collagenase activity, [^{14}C]glycine-labeled guinea pig skin collagen (≅10,000 dpm/mg, sp act) was used (15). A cold solution of [^{14}C]collagen (200 μl) containing 200 μg of collagen was reconstituted as fibrils at 37°C for 4–12 h in buffer containing 40 mM Tris HCl, pH 7.5, 120 mM NaCl, and 2.7 mM $CaCl_2$. 1 U of collagenase activity is defined as the amount required to solubilize 1 μg of reconstituted fibrils per min at 37°C. To detect enzyme in samples containing 10% FCS, collagenase activity was assayed in medium after prior treatment with trypsin (TRTPCK, Worthington Biochemical Corp., Freehold, N. J.), final concentration 200 μg/ml, for 10 min at 27°C with subsequent addition of a threefold weight excess of soybean trypsin inhibitor (Worthington Biochemical Corp.). An aliquot portion of this activated medium was then added to the collagen fibrils, mixed, and incubated for 1–24 h (depending upon the enzyme activity in each sample) at 37°C (11). The concentration of trypsin used in the present experiments was optimal for activation of latent collagenase in medium from unstimulated cells and cells stimulated with mononuclear cell culture medium. The shape of the curve showing dependence upon trypsin concentration for activation of latent collagenase in media from unstimulated cells was similar to that described (11).

Prostaglandin assay. Prostaglandins were measured in culture media as described (11, 15) by radioimmunoassay performed in Dr. Dwight R. Robinson's laboratory, Massachusetts General Hospital, with an antiserum to PGE_2 prepared by Dr. Lawrence Levine of Brandeis University (16).

Lysozyme assay. Lysozyme was assayed by lysis of heat-killed *Micrococcus lysodeikticus* incorporated into agarose. Test solutions (10 μl) were added to wells and the activity of lysozyme determined by measuring the diameter of the surrounding lytic zone (17) and comparing this to standard human lysozyme. The human lysozyme standard was a gift of Dr. Robert Canfield (Columbia University, New York).

Blood mononuclear cell preparation. Heparinized blood (10 U. S. Pharmacopeia U/ml) from normal volunteers was diluted with 3 vol of Hanks' balanced salt solution without calcium and magnesium (Grand Island Biological) containing Hepes, 12mM, pH ≅ 7.4. Aliquot portions of 35 ml of diluted blood were layered over 15 ml of Ficoll-diatrizoate (1.079, sp gr; Ficoll, Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) and the samples centrifuged at 2,000 *g* for 15 min at 4°C. Cells from the mononuclear layers were pooled, washed, and cultured in minimal essential medium containing 5% FCS, heat-inactivated and penicillin-streptomycin or processed further, depending on the experiment.

Fractionation of lymphocytes into nonimmunoglobulin-bearing and immunoglobulin-bearing populations. The mononuclear cell population was separated into surface, nonimmunoglobulin-bearing (Ig−) and surface immunoglobin-bearing (Ig+) cells by passage through Sephadex G-200 (Pharmacia Fine Chemicals Inc.) columns to which purified rabbit antihuman $F(ab)_2$ had been covalently coupled using cyanogen bromide (18). The cells passing through the column routinely contained fewer than 2% Ig+ lymphocytes estimated by immunofluorescence with a polyvalent fluoresceinated anti-Fab reagent. Essentially complete recovery of the column-bound Ig+ cells was achieved by competitive inhibition and elution with a 10% solution of human gamma globulin.

Monocytes were depleted from the lymphocyte population with the iron carbonyl technique or in the case of the Ig− cells by passage through nylon wool columns (19).

Preparation of monocyte-macrophages. Autologous adherent cells were obtained by overnight incubation of the unfractionated cells or cells passed through the anti-human $F(ab)_2$ immunoabsorbent column in plastic Petri dishes in culture medium (RPMI 1640, 2.5 mM Hepes buffer, 0.5% sodium bicarbonate, 200 mM l-glutamine, penicillin-streptomycin, supplemented with 20% heat-inactivated human AB serum). After extensive washing to remove the nonadherent cells, the adherent population was detached by brisk washing with cold serum-free minimal essential medium containing 2.5 mM EDTA. More than 90% of these adherent cells ingested latex particles and, by Wright-Giemsa staining, >98% had morphologic characteristics consistent with those of macrophages.

Production of collagenase-stimulating factor. The various mononuclear cell populations after separation and purification were incubated at 2×10^6 cells/ml in DMEM, 10% FCS

at 37°C in an atmosphere of 95% air, 5% CO_2 for 4 d with and without mitogens. Pokeweed mitogen (PWM; Grand Island Biological Co.) was used at a concentration of 10 μg/ml and phytohemagglutinin (PHA; Difco Laboratories, Detroit, Mich.) at 0.2 μg/ml. As described (13, 14), the supernatant medium was then removed after centrifugation of the cells, diluted (1:5–1:31) with fresh DMEM, 10% FCS, incubated with ASC in culture wells for 3–4 d, and the ASC supernatant medium assayed for collagenase and PGE_2.

Thymidine uptake. The thymidine uptake in response to soluble antigens was measured as described (18). In brief, 2×10^5 cells in 0.1 ml of culture medium were placed in flat bottom microtiter plates (Falcon Labware) and supplemented with 0.1 ml of culture medium with or without mitogen. Microplates were incubated in a 95% air, 5% Co_2 humidified atmosphere for 5 d and the cultures were then pulsed with 0.5 μCi of ^{3}H-labeled methyl thymidine (1.9 Ci/mmol, sp act; Schwartz/Mann Div., Becton, Dickson & Co., Orangeburg, N. Y.). After a 16-h pulse, the cells were harvested using a Mash II apparatus and the [^{3}H]thymidine incorporation measured in a Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Results for triplicate samples were expressed as mean counts per min±SEM.

RESULTS

Evidence that the monocyte population is the predominant cell producing collagenase- and PGE_2-stimulating factor. In initial experiments unfractionated mononuclear cells were compared to adherent monocytes as well as to highly purified Ig− (T) and Ig+ (B) cells for the capacity to secrete collagenase- and PGE_2-stimulating factor in cell culture. Mononuclear cell preparations obtained from Ficoll-diatrizoate gradients were permitted to adhere to the surface of plastic Petri dishes. The nonadherent cells were then purified in two stages. First, they were further depleted of monocytes by iron carbonyl treatment. Next, they were separated into Ig+ and Ig− fractions on Sephadex G-200 anti-human $F(ab)_2$ immunoabsorbent columns. The four populations thus separated (unfractionated, adherent, Ig+, and Ig−) were then incubated with or without lectin for 3 d and the supernatant media from these cultures were diluted, and incubated with ASC for 3 d. The ASC culture media were then assayed for collagenase and PGE_2 content. In all experiments simultaneous measurement of lysozyme production by the peripheral blood mononuclear cells was performed to estimate the level of monocyte contamination in each fraction. The results of a representative experiment are shown in Fig. 1. As can be seen, culture media from the monocyte population purified in this experiment by adherence of the unfractionated mononuclear cell population accounted for the bulk of collagenase-stimulating factor production. Significant stimulating activity was found in media from both unfractionated populations and purified B lymphocyte populations. We emphasize, however, that the latter populations produced significant levels of lysozyme activity. In contrast, highly purified T lymphocytes with

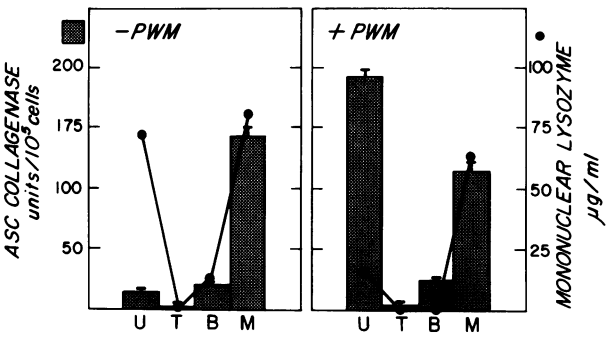

FIGURE 1 Production of collagenase-stimulating activity and lysozyme by peripheral blood mononuclear cells. ASC in the second passage were plated at 1×10^5/well 5 d before bioassay. ASC were then incubated for 3 d with media from unfractionated lymphocytes and monocytes (U), Ig− cells (T) and Ig+ cells (B) and monocytes (M) purified by 24 h adherence from the unfractionated population. Mononuclear cells were incubated without (−PWM) or with (+PWM) 10 μg/ml PWM. Media were then diluted 1:11 with DMEM, 10% FCS before incubation with ASC. Bars indicate collagenase production by ASC exposed to the various supernatant media. Values shown are mean±SEM for three wells. Collagenase activity in unstimulated ASC was 0.3±0.1 U/10^5 cells. Values of lysozyme measured in undiluted media from the cultured mononuclear cell populations are indicated by closed circles.

negligible lysozyme activity did not produce significant collagenase-stimulating activity. These initial experiments thus suggested that the monocyte population was a major source of the collagenase-stimulating factor in unfractionated cells. The T cells produced no stimulating factor and although the B cells did, the presence of lysozyme in the supernatants of their culture media suggested significant monocyte contamination. Furthermore, in an experiment such as that depicted in Fig. 2 in which no lectin was added, the amount of collagenase-stimulating activity was proportional to the density of adherent monocyte-macrophages, the latter estimated by cell number or functionally by lysozyme production.

In previous studies, it was shown that lectins could modulate the amount of collagenase- and PGE_2-stimulating factor produced by mononuclear cell populations (13, 14). To determine the effect of lectins on more purified populations, each of the cell populations shown in Fig. 1 was triggered with the lectin PWM and then assayed for both collagenase-stimulating activity and lysozyme production. PWM had a markedly enhancing effect on the production of collagenase-stimulating factor in unfractionated populations. In contrast, PWM triggering of purified T, B, or monocyte populations did not enhance collagenase-stimulating activity (Fig. 1). The enhancing effect of PWM on the unfractionated mononuclear cell population could not be accounted for by direct stimulation of monocytes within the unfractionated population, since PWM had

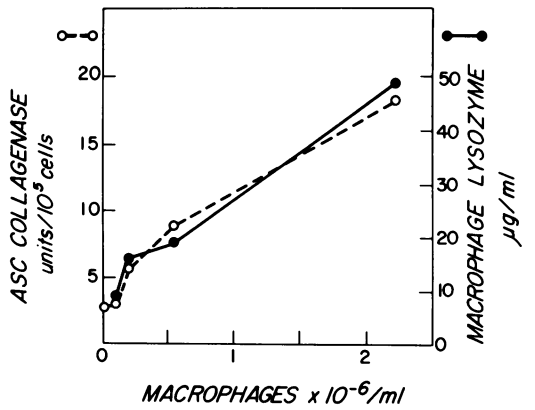

FIGURE 2 Collagenase-stimulating activity and lysozyme production by purified peripheral blood macrophages. ASC in the first passage were plated at 1×10^5/well 6 d before the 3-d incubation with media from monocyte-macrophages obtained by overnight adherence to plastic dishes. Monocytes were then cultured at various densities for 3 d and the supernatant media diluted 1:10 with DMEM, 10% FCS before adding to ASC. Open circles (○) indicate collagenase production by ASC exposed to the diluted supernatant media from monocytes cultured at indicated concentrations. Closed circles (●) indicate lysozyme activity in undiluted supernatant media from cultured monocytes.

no direct stimulatory effect on isolated monocyte-macrophage populations alone with respect to collagenase-stimulating factor. Taken together, these results suggest that cell-cell interactions within the unfractionated populations were triggered by PWM with the end result being enhanced production of collagenase-stimulating activity.

To examine further which cell is responsible for production of collagenase-stimulating activity as well as PGE_2-stimulating activity, experiments were designed using ASC at the same passage from two different rheumatoid patients for bioassay of mononuclear cell culture media. For these experiments (Table I) we were fortunate to have two different synovectomy specimens for culture on the same day. As shown in Table I, only monocyte-macrophage supernates stimulated collagenase and PGE_2 production significantly in either of the two synovial cell cultures. The magnitude of stimulation was similar and the presence of PHA had no effect on the mononuclear cell stimulating activity. Since the mononuclear cell media were diluted 1:20 for incubation with synovial cells, the contribution of mononuclear cell PGE_2 (2.1 and 2.8 ng/ml) was insignificant compared to that produced by the synovial cells exposed to the mononuclear cell media (123.5–164.9 ng/ml). Furthermore, in other experiments (not shown) in which PGE_2 production by monocytes was inhibited by indomethacin (14 µM) >95%, there was no change in collagenase-stimulating activity. Collagenase activity was not detectable in any cultures of any population of peripheral blood mononuclear cells in the presence or absence of serum, with or without trypsin activation.

Evidence for cell-cell interactions in the generation of collagenase-stimulating activity. In the experiments presented so far, the effect of lectins on the production of collagenase- or PGE_2-stimulating activity appeared to be limited to the unfractionated mononuclear cells. Thus, while PWM markedly enhanced production of the factor by unfractionated populations, it

TABLE I

Production of Collagenase- and PGE_2-stimulating Activity by Purified Peripheral Blood Monocytes and T Lymphocytes

Peripheral blood mononuclear cells						Adherent synovial cells			
Cell						Collagenase		PGE_2	
Population	Density	PHA	[³H]Thymidine incorporation	Lysozyme	PGE_2	ASC-123	ASC-124	ASC-123	ASC-124
	$\times 10^{-6}$/ml	µg/ml	cpm	µg/ml	ng/ml	U/10⁶ cells		ng/10⁶ cells	
M	1.0	0	398±77	13	42.5	42.1±11.5	63.5±4.4	819.2±66.5	824.3±286.1
	1.0	0.2	732±121	14	76.1	45.7±11.2	70.6±10.0	801.3±143.4	617.3±217.7
T	1.0	0	62±21	<1	<0.4	0.2±0.1	0.4±0.3	1.7±0.1	1.5±0.2
	1.0	0.2	11,955±857	<1	<0.4	0.1±0.1	0.6±0.5	2.0±0.1	1.8±0.3
None	—	—	—	—	—	0.1±0.1	0.1±0.1	2.1±0.2	2.5±0.2

Cell cultures (ASC-123 and ASC-124) were prepared on the same day from synovectomy specimens from two different subjects with rheumatoid arthritis, and for this experiment, plated in the second passage at 1×10^5 cells/well 5 d before assay. ASC were then incubated for 3 d with media from the same cultures of purified peripheral blood monocytes (M) or purified Ig− cells (T). The mononuclear cell media were diluted 1:20 with DMEM, 10% FCS, before adding to ASC. Lysozyme and PGE_2 were measured in undiluted media from M and T. Basal collagenase activity and PGE_2 levels in ASC-123 and ASC-124 were 0.1±0.1 and 0.1±0.1 U/10⁶ cells and 2.1±0.2 and 2.5±0.2 ng/10⁶ cells, respectively. Levels of collagenase and PGE_2 production by ASC incubated with PHA alone were not different from those in ASC not incubated with PHA.

had no significant effect on factor production by purified populations of either T cells, B cells, or monocytes. These studies suggested that cell-cell interactions within the unfractionated population may have accounted for the enhanced activity of this population after triggering by PWM. To address this question in more detail, adherent cells were separated from lymphocytes and then added back to Ig− and Ig+ cells that had been partially depleted of monocytes by iron carbonyl treatment (Table II). The adherent population alone produced the greatest amounts of lysozyme as well as collagenase- and PGE_2-stimulating activity. The amounts of PGE_2 produced by the mononuclear cells alone were low and collagenase undetectable in mononuclear cell media. Isolated T cell populations, as above, produced no detectable lysozyme and very low levels of collagenase- and PGE_2-stimulating activity with or without PWM. That these T cells retained functional activity is shown by their capacity to proliferate in response to PWM. Interestingly, when monocytes were added back to the T cells (5% monocytes and 95% T cells) there was little augmentation of collagenase- and PGE_2-stimulating factor production unless PWM was added. Under the latter conditions, increased levels of collagenase- and PGE_2-stimulating activity were obtained that approached those of the monocyte-macrophages alone. Similar findings were observed when monocyte-macrophages were added back to B cell populations, although these experiments were more difficult to interpret because B cell cultures alone produced a significant amount of lysozyme. Production of lysozyme by the mononuclear cells was usually decreased in the presence of PWM.

DISCUSSION

The results of these studies confirm and extend our previous observations showing that products of human peripheral blood mononuclear cells release in cell culture a soluble factor that can stimulate the production of collagenase and PGE_2 by adherent rheumatoid synovial cells (13, 14). The present studies were undertaken to determine more precisely the predominant cell type responsible for the production and release of this factor and whether cell-cell interactions are important in its regulation. The following points emerge from these studies: (*a*) adherent monocyte populations, largely devoid of lymphocytes, are the predominant populations in the peripheral blood producing collagenase- and PGE_2-stimulating factor; (*b*) highly purified T lymphocytes isolated from anti-human F $(ab)_2$ immunoabsorbent columns secrete little if any of this factor; (*c*) Ig+ (B-cell) populations do release stimulating activity, although the presence of lysozyme in culture supernates from these cells sug-

TABLE II

Properties of Partially Purified Peripheral Blood Mononuclear Cell Preparations and Some Effects of their Culture Media on Adherent Rheumatoid Synovial Cells

Peripheral blood mononuclear cells						Adherent synovial cells			
Cell						Collagenase		PGE_2	
Population	Density	PWM	[^{3}H]Thymidine incorporation	Lysozyme	PGE_2	1:11	1:31	1:11	1:31
	×10⁻⁶/ml	*μg/ml*	*cpm*	*μg/ml*	*ng/ml*	*U/10⁶ cells*		*ng/10⁶ cells*	
U	2.0	0	ND	72	0.8	12.5±1.4	0.4±0.1	330±36	132±6
		10	ND	15	<0.4	192.9±6.5	35.3±2.1	2,637±504	681±39
M	2.0	0	6,527±2,330	82	4.0	142.3±8.9	7.5±1.3	1,215±36	372±60
		10	5,571±779	63	2.0	114.9±7.9	13.1±0.9	1,185±129	408±81
T	2.0	0	1,143±823	0	<0.4	1.6±0.1	0.2±0.1	33±6	21±3
		10	22,210±1,374	0	<0.4	4.0±0.5	0.3±0.1	189±48	42±3
T + M	1.9 (T) + 0.1 (M)	0	849±150	24	<0.4	2.6±0.4	0.4±0.1	240±51	48±3
		10	124,815±9821	0	<0.4	92.1±10.2	8.4±1.4	2,640±30	837±321
B	2.0	0	924±424	12	<0.4	18.3±0.7	0.7±0.1	504±123	131±36
		10	32,439±924	0	<0.4	23.1±1.4	1.6±0.1	714±177	171±15
B + M	1.9 (B) + 0.1 (M)	0	3,001±1,155	15	<0.4	54.9±4.1	1.7±0.4	690±30	251±72
		10	39,709±5,406	0	<0.4	114.8±3.3	7.1±0.9	1,257±102	384±84
	None	—	—	—	—	0.3±0.1		34±9	

Synovial cells in the second passage were plated at 1 × 10^5 cells/well 5 d before assay. ASC were then incubated for 3 d with media from unfractionated lymphocytes and monocytes (U), monocytes (M), Ig− cells (T), and Ig+ cells (B). Media were diluted at 1:11 and 1:31 with DMEM, 10% FCS before adding to ASC. Lysozyme and PGE_2 were measured in undiluted media from U, M, T, and B. Collagenase activity and PGE_2 levels were 0.3±0.1 U/10^6 cells and 34±9 ng/10^6 cells in unstimulated ASC, respectively. Each value is the mean±SEM for three wells. Levels of collagenase and PGE_2 production by ASC incubated with PWM alone were not different from those in ASC not incubated with PWM.

gests significant monocyte contamination; and (*d*) T-cell populations, although incapable themselves of producing collagenase- and PGE_2-stimulating activity, do modulate the production of stimulating activity by monocyte-macrophages.

The subpopulations of lymphocytes and adherent cells examined in these studies were enriched with respect to certain functional properties and surface markers but cannot be considered pure cell populations. The T-cell population, which was relatively free of monocytes and B cells (<2% Ig+ cells), produced little detectable collagenase- or PGE_2-stimulating activity. Since the sensitivity of the response to stimulating activity varies with different sources and preparations of the target synovial cells, it cannot be stated conclusively that the T cells produce no such activity. However, it is clear that the levels produced by T-cell populations alone could only be a small percentage of those produced by the most highly enriched monocyte-macrophage cell populations.

The monocyte-macrophage population consistently produced collagenase- and PGE_2-stimulating activity, even in the absence of triggering with lectins, and the amount produced was proportional to the number of cells. The apparent molecular weight of the factor produced by these adherent cells, estimated by gel filtration (experiments not shown), is similar to that produced by the unfractionated mononuclear cell population as previously reported and is in the range of 14,000 daltons (13, 14). Consistent with generally recognized properties of these cells, there was little mitogenic response to lectin. These monocytes, however, produced significant amounts of lysozyme which served in our experiments as a convenient marker to detect their presence. The observation that PWM consistently decreased lysozyme production is of interest, although the nature of this inhibition has not been investigated further as yet.

The Ig+ cell populations isolated from the anti-human $F(ab)_2$ columns, even after removal of adherent cells and phagocytic cells by iron carbonyl, still produced significant quantities of collagenase- and PGE_2-stimulating activity. However, supernates from these Ig+ populations contained significant amounts of lysozyme activity. Because the levels of lysozyme produced by these Ig+ cells were consistent with the presence of 5–10% monocyte-macrophage contamination, and because 5% monocyte-macrophages could produce the assayable collagenase- and PGE_2-stimulating activity, it is uncertain whether the B cells alone produce such activity. Nevertheless, quantitatively most of the stimulating activity in Ig+ cells could be accounted for by contaminating macrophages. In some experiments, the level of stimulating activity in Ig+ populations could be increased in the presence of PWM. These data suggest that some cellular interactions between Ig+ cells and monocytes may play a role in the production of stimulating activity because monocyte-macrophage populations alone were not affected by lectins. Furthermore, the contamination of B cell populations by relatively small numbers of monocyte-macrophages, detectable by measurement of lysozyme production, raises caution in the interpretation of other reported functional activities of isolated Ig+ cells (20–22). The evidence for lymphocyte-monocyte interactions in the production of collagenase- and PGE_2-stimulating activity is perhaps more convincing with respect to T lymphocyte-monocyte interactions. For example, when the mononuclear cell density was kept constant at 2×10^6/ml, the addition of 5% monocytes to highly purified T-cell populations, the latter inactive by themselves, resulted in increased collagenase- and PGE_2-stimulating activity. This effect was dramatically augmented by PWM. These interactions are undoubtedly complex. One can envision a model in which T lymphocytes triggered by mitogens or antigens may release soluble molecules that subsequently trigger the release of stimulating activity from monocytes, although we have no direct evidence for such soluble T lymphocyte products in our system.

The cells dispersed from the rheumatoid synovial lining which produced collagenase in vitro in our system do not themselves have macrophage characteristics such as Fc receptors or lysozyme production (11). However, these synovial cells produce prostaglandins, particularly PGE_2, in relatively high amounts. Although PGE_2 can modulate collagenase production by rodent macrophages (23), as well as by human rheumatoid synovial cells (24), the amount of PGE_2 in the mononuclear cell media used in the present studies cannot account for the observed effects on collagenase production by synovial cells, and monocytes incubated in the presence of indomethacin still release collagenase-stimulating factor (25).

Although the cultured adherent rheumatoid synovial cells that produce collagenase and PGE_2 do not possess known morphological or functional characteristics of macrophages, they can respond to products of monocyte-macrophages, at least the peripheral blood monocyte-macrophages examined in the present studies. This is most dramatically seen in the effect of macrophage products on the levels of collagenase and PGE_2. On the other hand, the typical macrophages produce no collagenase and only low levels of PGE_2. We would emphasize, however, that it may not be the macrophage alone that ultimately controls production of mononuclear cell factors, because further stimulatory effects can be observed with the addition of lectin-triggered lymphocytes. The presence of all of these interacting mononuclear cells in the inflammatory lesion and their release of soluble mediators that affect

other cells may explain certain characteristics of the rheumatoid lesion, such as destruction of the collagenous connective tissue components. Whether mononuclear cells with macrophage markers from the local synovial lesion or peripheral blood cells from patients with rheumatoid arthritis produce amounts of collagenase- or PGE_2-stimulating activity similar to those produced by normal peripheral blood cells will be the subject of further investigation. It is clear from our present studies that the use of purified subpopulations of mononuclear cells may be important in the interpretation of these clinical investigations.

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