Human Mononuclear Cell Factors Mediate Cartilage Matrix Degradation through Chondrocyte Activation

HUGO E. JASIN and JOHN T. DINGLE, *Tissue Physiology Department, Strangeways Research Laboratory, Cambridge, England; Rheumatic Diseases Unit, Department of Internal Medicine, Southwestern Medical School, Dallas, Texas 75235*

ABSTRACT Human blood mononuclear cells (BMC) in short-term culture secrete one or more factors that induce degradation of matrix proteoglycan and collagen in cartilage explants in organ culture. Induction of matrix degradation took place both in nasal septum and articular cartilage explants in the presence of the mononuclear cell supernates. Cartilage degradation in this system was absolutely dependent on the presence of live chondrocytes. Matrix depletion did not occur in dead cartilage explants cultured with active supernates. Supernates obtained from unstimulated BMC showed variable cartilage matrix degrading activity (MDA). BMC stimulated with phytohemagglutinin (PHA) showed increased MDA, which in one dilution experiment was found to be five times higher than that in the unstimulated control supernate. Concanavalin A and pokeweed mitogen were also shown to stimulate release of MDA. Time experiments showed that most of the degrading activity was released by the mononuclear cells during the first day of culture.

The cellular origin of MDA was investigated with the aid of partially purified BMC subpopulations. Removal of adherent cells resulted in a decrease of MDA release. Purified T lymphocytes failed to show enhanced MDA release in spite of their ability to mount a virtually intact proliferative response to PHA. Purified adherent cells also failed to show enhanced PHA-dependent MDA release. Nevertheless, restoration of PHA-dependent MDA release took place in reconstituted cell populations containing both T lymphocytes and monocytes. These experiments suggest that MDA may be released by adherent mononuclear cells, presumably monocytes, and that the PHA-dependent increase in MDA release may be mediated by T lymphocytes.

Partial characterization of MDA by gel chromatography showed one active fraction corresponding to an apparent molecular weight ranging from 12,000 to 20,000. The fraction was also shown to degrade cartilage matrix only in the presence of live chondrocytes. These results demonstrate that factors released by human BMC mediate degradation of matrix proteoglycan and collagen in intact cartilage explants through chondrocyte activation. This pathogenic mechanism may play a role in in vivo cartilage destruction in chronic inflammatory joint diseases.

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INTRODUCTION

The processes that lead to irreversible articular cartilage destruction in inflammatory and degenerative joint diseases are mostly unknown. Although several enzymatic mechanisms such as the synovial fluid proteases of diverse origin and enzymes derived from pannus cells and activated chondrocytes have been postulated to play a role in cartilage degradation (1–3), direct evidence implicating a particular mechanism is not available. Although proteases capable of breaking down cartilage matrix components are present in pathologic synovial fluids, their role in cartilage destruction has not been resolved.

An alternative hypothesis implicating the stimulated chondrocyte as one of the cells responsible for cartilage matrix degradation has been suggested recently. The postulated mechanism was developed from the demonstration of Fell and Jubb (4) that live synovial tissue induced cartilage matrix depletion when co-cultured in the same dish not in contact with live cartilage explants, whereas no depletion was seen with dead cartilage. Subsequent work demonstrated a factor secreted by synovial tissue cells that induced matrix breakdown in explants containing living chondrocytes (5). Steinberg et al. (6) were able to reproduce Fell and Jubb's findings using rheumatoid synovium and bovine nasal cartilage explants. These authors showed that although matrix degradation took place in cultures of synovium over-

lapping with dead cartilage, optimal proteoglycan release required interaction of living synovium with live cartilage. A related group of investigations have dealt with factors secreted by mononuclear cells that induce secretion of collagenase and neutral proteases from chondrocytes (7–9), rheumatoid synovial cells (10), fibroblasts (11), and macrophages (12) in culture. It was postulated that the increased production of these enzymes in vivo may mediate cartilage degradation, but there has been no direct evidence that connective tissue cells stimulated by such factors are able to mediate matrix degradation in living cartilage. The present work demonstrates for the first time that factors secreted in vitro by human blood mononuclear cells (BMC)[1] induce degradation of matrix proteoglycan and collagen in cartilage explants in organ culture. Cartilage degradation in this system is dependent on the presence of live chondrocytes.

METHODS

Blood mononuclear cell cultures. Heparinized blood obtained from normal volunteers was diluted with 1 vol of sterile phosphate-buffered saline, pH 7.4. The mononuclear cells were separated by centrifugation on Ficoll-sodium ditrizoate cushions (Ficoll-Paque, Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Hounslow, Middlesex, England) according to the method of Böyum (13). The mononuclear cells were washed twice in Dulbecco's modification of minimal essential medium (DMEM, Grand Island Biological Co., Uxbridge, Middlesex, England) and once in DMEM containing 10% fetal calf serum (Grand Island Biological Co.) heated at 56°C for 30 min. The cell suspensions were cultured for 48 h at 37°C in 95% air, 5% CO_2, in 25-ml capacity, conical bottom, plastic universal vials (Sterilin Ltd., Richmond, Surrey, England). Each culture vial contained 2 ml of cell suspension, at a concentration of 2×10^6 cells/ml. In some experiments, phytohemagglutinin (PHA, Wellcome Reagents Ltd., Beckenham, England) was added at a concentration of 10 μl/ml, concanavalin A (Pharmacia Fine Chemicals, Piscataway, N. J.) at 10 μg/ml, and pokeweed mitogen (Grand Island Biological Co.) at 10 μl/ml. At the end of the culture period, the cells were centrifugated at 600 *g* for 5 min and the cell-free supernates were collected. Identical amounts of mitogen used in any particular experiment were added to the control (unstimulated supernates), and then stored at −20°C. Preliminary experiments had shown that addition of PHA to culture medium alone did not modify base-line proteoglycan release from the cartilage explants.

Mononuclear cell fractionation. Partial purification of T lymphocytes and nonadherent mononuclear cells (NAC) was carried out by two different methods. NAC were obtained after 1-h incubation steps of the original BMC in 30-mm Diam plastic petri dishes at 37°C to eliminate adherent cells. T lymphocytes were purified by rosetting the BMC with neuroaminidase-treated sheep erythrocytes according to the method of Keightley et al. (14). To identify phagocytic cells the BMC were incubated at 37°C for 30 min with polystyrene spheres of 0.81 μm Diam (Difco Laboratories, Detroit, Mich.) before rosetting. After separation by centrifugation over Ficoll-Hypaque cushions, the cell button was washed twice with Hank's solution and the erythrocytes were lysed with ice-cold NH_4Cl buffer. The T lymphocyte population contained from 0.5 to 2% monocytes identified by the presence of two or more ingested polystyrene spheres. To obtain adherent mononuclear cells, the nonrosetted cells at the Ficoll-Hypaque interphase were washed and incubated on glass petri dishes for 1 h at 37°C in medium containing 10% fetal calf serum. The NAC were removed by repeated washings with culture medium. The petri dishes were further incubated for 1 h at 4°C in phosphate saline, pH 7.4. The adherent cells were then gently detached with a rubber policeman, washed, and adjusted to a concentration of 0.5 to 2×10^6 cells/ml. These populations contained from 82 to 90% phagocytic cells. The purified T lymphocytes and monocytes were also cultured together in a 3:1 ratio at a concentration of 2×10^6/ml.

The proliferative response of the mononuclear cells after 48 h in culture was measured in some experiments. After removal of the supernates to be assayed for matrix degrading activity (MDA), 2 ml of fresh culture medium containing 1 μCi/ml, 6.7 Ci/mmol [^{3}H]thymidine (New England Nuclear, Boston, Mass.) were added to each tube containing originally 4×10^6 cells. After incubation for 18 h, the cells were washed three times with normal saline solution and once with 10% trichloroacetic acid solution. The pellets were dissolved in 0.1 N NaOH and the radioactivity determined in a Beckman liquid scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif.).

Biological assays of MDA. Articular cartilage explants were obtained from the metacarpophalangeal joints of 5–9-mo-old pigs slaughtered within 2 h (4). Paired explants measuring 2 mm wide × 5–8 mm long were cultured for 12 d over stainless steel grids in 30-mm plastic petri dishes containing 1.5 ml DMEM, 10% fetal calf serum, 15 mg/dl ascorbic acid, and the cell-free supernates at 20% concentration. The culture medium was changed every 4 d and stored at −20°C until assayed for release of proteoglycan and hydroxyproline, as described below. At the end of the culture period, the tissue explants were fixed and stained with toluidine blue for proteoglycan and with van Gieson for collagen (5, 15). Independent semi-quantitative histology assessment of matrix depletion was carried out according to Fell and Jubb (4) by two observers (Dame Honor Fell and H. E. Jasin).

A second assay for cartilage breakdown, suitable for the determination of factors inducing cartilage matrix depletion in a large number of specimens, involved the use of bovine nasal septum cartilage (5). Cartilage disks free of perichondrium, obtained from 1-yr-old cows, and measuring 4 mm in Diam × 1–2 mm thick were cultured for 8 d at 37°C in 30-mm plastic petri dishes (Sterilin Ltd.) with 1 ml DMEM–5% fetal calf serum containing the mononuclear cell supernates at a concentration of 10%, unless specified otherwise in Results. The culture media from each dish were changed once on day 4. Each sample was assayed in quintuplicate. Also included were negative controls containing culture medium alone and positive controls containing an excess of cartilage catabolic factor derived from pig synovium (catabolin) (5) to determine chondrocyte-mediated maximum proteoglycan degradation for each experiment. In some experiments the explants were killed before culture by freezing and thawing three times. At the end of the culture period the cartilage disks were digested with papain (15), and both the tissue digests and culture supernates collected at days 4 and 8 were assayed for chondroitin sulfate content by a colori-

[1] *Abbreviations used in this paper*: BMC, blood mononuclear cell(s); DMEM, Dulbecco's minimal essential medium; MDA, matrix degrading activity; NAC, nonadherent cells; PHA, phytohemagglutinin.

metric method (16) using 1,9-dimethyl methylene blue (Serva Feinbiochemica, Gmbh & Co., Heidelberg, West Germany) in sodium formate buffer, pH 3.5. Standard curves were constructed with purified shark chondroitin sulfate (Koch-Light Laboratories Ltd., Colnbrook Bucks, England.). The results were expressed as the cumulative percentage of total chondroitin sulfate released on days 4 and 8, (micrograms chondroitin sulfate in supernate × 100)/micrograms chondroitin sulfate in supernates + tissue). Maximal proteoglycan release induced by excess pig synovium factor (5) averaged 37.7±3.8% (SEM) on day 4 and 80.2±3.2% on day 8 of culture in 11 separate experiments.

Quantitative assessment of MDA concentrations in BMC supernates was achieved by assaying MDA at several concentrations. The degrading activity was expressed in arbitrary units calculated from the inverse of the concentration, which yielded 50% proteoglycan release. In the experiment depicted in Fig. 2, the unstimulated BMC supernate contained 5 MDA units and the PHA-stimulated BMC contained 25 MDA units.

Cartilage collagen breakdown was measured by analysis of hydroxyproline in the supernate (17, 18) previously hydrolyzed in 6 N HCl at 105°C for 20 h.

Gel filtration chromatography. Culture supernates obtained from PHA-stimulated mononuclear cell cultures were dialyzed against DMEM diluted 10-fold with water. The dialyzed samples were freeze-dried and reconstituted with distilled water at 10 times the original concentration. 2-ml samples corresponding to 20 ml of the original supernates were applied to a 1.6 × 110-cm column packed with Ultrogel AcA 44 (LKB Instruments, Inc., Surrey, England) equilibrated with phosphate-buffered saline, pH 7.4. 4-ml fractions were collected at 4°C and pooled into seven fractions of 20 ml each. The pooled fractions were dialyzed against basal salts solution of DMEM at 4°C, sterilized by micropore filtration, and tested for MDA at 10% concentration by the bovine nasal cartilage assay.

RESULTS

All PHA-stimulated mononuclear cell supernates obtained from nine different donors showed nasal cartilage degrading activity when tested at 10% concentration. The mean percentage release of matrix proteoglycan induced by unstimulated and PHA-stimulated supernates is shown in Table I. Supernates obtained from unstimulated cell cultures showed variable activity at low concentrations, and MDA was always lower than the activity showed by the corresponding supernate obtained from PHA-stimulated cells. When the mononuclear cell supernates were tested at 50% concentration, proteoglycan release from the nasal cartilage disks was maximum, both with the supernates obtained from unstimulated and PHA-stimulated cells (Fig. 1A). As these were diluted to 25 and 10% concentration, however, the supernates derived from unstimulated cells showed decreasing activity, whereas the media obtained from PHA-stimulated cells continued to show near-maximum MDA even at 10%

TABLE I

Mean Percentage Release of Matrix Proteoglycan Induced by Unstimulated and PHA-stimulated Supernates

Supernate	Chondroitin sulfate release	
	Day 4	Day 8
	%±SEM	
Medium	13.0±1.2	26.6±2.0
Unstimulated	16.2±1.9	42.6±6.6
PHA-stimulated	35.3±4.3	79.8±1.6

Mononuclear cell supernates from nine separate donors incubated with nasal cartilage disks were used at 10% concentration.

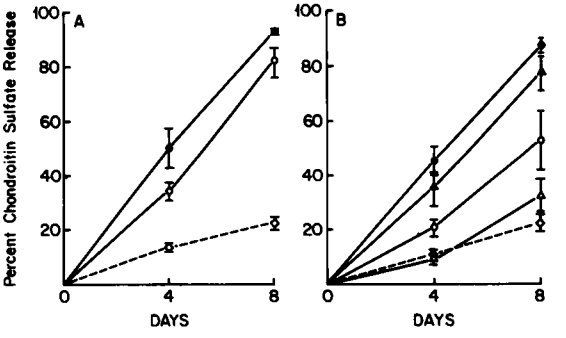

FIGURE 1 Proteoglycan release from bovine nasal cartilage explants cultured with supernates obtained from unstimulated and PHA-stimulated BMC. (A) Supernates used at 50% concentration. ○, unstimulated; ●, PHA-stimulated; ◇, control, culture medium alone. (B) ●, PHA-stimulated 25% concentration; ▲, PHA-stimulated 10%; ○, unstimulated 25%; △, unstimulated 10%; ◇, culture medium. Brackets represent SEM.

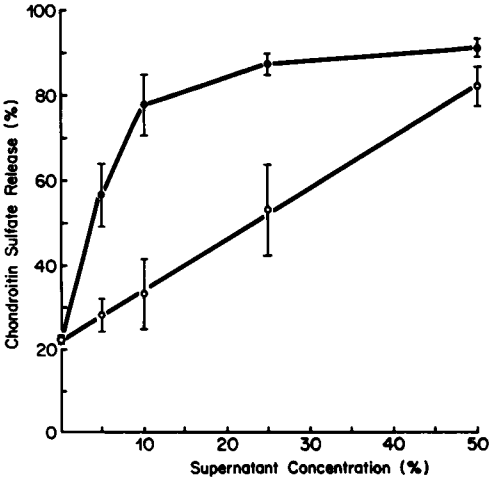

FIGURE 2 Concentration dependence of proteoglycan release fom bovine nasal cartilage explants on day 8 of culture induced by supernates from unstimulated and PHA-stimulated BMC. ○, unstimulated BMC supernate; ●, PHA-stimulated BMC supernate; ◇, culture medium control. The unstimulated supernate contained 5 MDA U, and the PHA-stimulated supernate contained 25 MDA U.

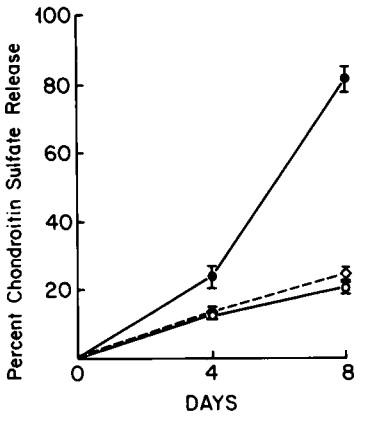

FIGURE 3 Proteoglycan release from live and dead nasal cartilage explants cultured with active BMC supernates. ●, live cartilage cultured with PHA-stimulated BMC supernate; ○, dead cartilage cultured with PHA-stimulated BMC supernate; ◇, live cartilage with culture medium alone. Supernate was used at 10% concentration.

concentration (Fig. 1B). When the MDA measured on day 8 of culture was plotted against supernatant concentration, a linear dose-response curve was obtained for the unstimulated supernate diluted from 5 to 50%. The PHA-stimulated supernate also showed a linear response at concentrations yielding below maximal MDA (Fig. 2). It was apparent from these dilution experiments that PHA-stimulated cells produced roughly five times more MDA than the unstimulated cell controls. On the basis of these results, it was decided to test the supernates at 10% concentration.

To rule out the possibility that proteolytic enzymes released by the cultured mononuclear cells may have been directly responsible for cartilage degradation, most experiments included a set of dead cartilage disks. The experiment depicted in Fig. 3 shows the absolute dependence of MDA on the presence of viable chondrocytes. Whereas proteoglycan released from the live cartilage disks was >80% when cultured with a supernate derived from PHA-stimulated cells, release was no different from control levels in dead explants cultured with the same supernate.

The mononuclear cell supernates were also tested for their capacity to induce degradation of articular cartilage. In the experiment shown in Fig. 4, the supernates were used at 20% concentration for incubation with quadruplicate articular cartilage explants. Since the tissues were processed for histologic examination at the end of the culture, the results could not be expressed as the percentage of total proteoglycan and collagen released; they are instead expressed in cumulative amounts of chondroitin sulfate and hydroxyproline released per milligram of wet tissue. It can be seen that MDA was readily detectable in the cultures containing supernates from PHA-stimulated cells, both for release of proteoglycan and collagen. The total amount of collagen released in the supernate at the end of the culture period was estimated to be nearly 50% of the total amount of collagen in the explants. As was previously shown for nasal cartilage, significant MDA was not elicited with frozen and thawed explants.

These results correlated well with the histologic assessment of articular cartilage matrix depletion (Table II). Fig. 5B shows the complete disappearance of metachromatic staining from articular cartilage cultured in the presence of active supernates, as compared with a similar explant cultured with control medium alone (Fig. 5A), which showed little or no evidence of matrix depletion. The explants exposed to supernates derived from unstimulated mononuclear cells showed slight-to-moderate proteoglycan depletion. The differences observed for collagen depletion assessed by the van Gieson stain were even more substantial. No evidence of depletion was seen in the explants cultured with medium or control supernates (Fig. 5C), whereas the explants exposed to PHA-stimulated cell supernate showed from moderate (Fig. 5D) to complete disappearance of stainable collagen. Moreover, the explants exposed to the active supernates showed a significant decrease in thickness. These results are in agreement with the biochemical evidence of depletion shown in Fig. 4. Semiquantitative histologic assessment of matrix proteoglycan and collagen depletion in the quadruplicate articular cartilage explants is shown in Table II.

Increased MDA was also demonstrated in mononuclear cell cultures stimulated with other lymphocyte mitogens such as concanavalin A and pokeweed mitogen. Table III shows the degree of matrix depletion induced in bovine nasal cartilage disks exposed

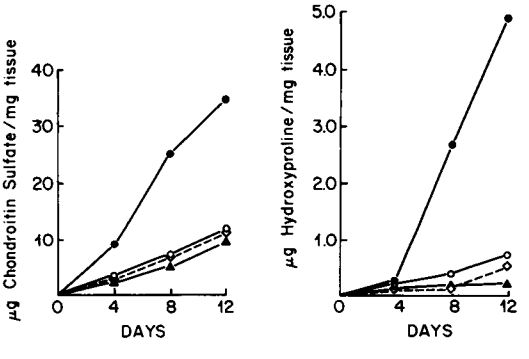

FIGURE 4 Articular cartilage matrix degradation by BMC supernates in organ culture. Left: Matrix proteoglycan release. Right: Collagen release. ●, PHA-stimulated BMC supernate; ○, unstimulated BMC supernate; ▲, dead cartilage cultured with PHA-stimulated BMC supernate; ◇, culture medium alone. Results represent the mean of two pairs of explants cultured in two dishes. Supernates were used at 20% concentration.

TABLE II
Histologic Assessment of Articular Cartilage Matrix Depletion

Supernate	Depletion of	
	Proteoglycan (toluidine blue)	Collagen (Van Gieson)
Medium	±, +, ±, +	−, −, −, −
Control	+, ++, ++, +	−, −, −, −
PHA-stimulated	++++, ++++, ++++, ++++	++, +++, ++, ++++

Semiquantitative assessment of matrix proteoglycan and collagen depletion was carried out on serial sections of quadruplicate cartilage explants according to Fell and Jubb (4).

to these supernates. Both mitogens increased the MDA released by BMC, when compared with similar supernate obtained from unstimulated cells.

The time of appearance of MDA in the mononuclear cell culture supernates was investigated (Table IV). Supernates harvested after 24 h of culture showed virtually identical MDA as the culture media collected on days 2 and 3, which suggests that most of the activity was released by the mononuclear cells during the 1st d of culture.

To rule out the possibility that the above-mentioned results were due to MDA degradation in culture, a second experiment was performed in which the culture medium was changed at frequent intervals. The PHA-stimulated supernate obtained at 0–4, 4–8, 8–16, 16–24, and 24–48 h of culture were assayed for MDA and compared with a similar supernate obtained from culture tubes left undisturbed for 48 h. The results shown in Table V demonstrate that most of the MDA was released by the BMC in the first 16 h of culture, which confirms the results obtained in the previous experiment.

The cellular origin of MDA was also investigated in a series of experiments using partially purified BMC subpopulations. When adherent cells were removed from BMC by two incubation steps on plastic surfaces, PHA-dependent enhancement of MDA release was uniformly abolished, as is shown in Table VI. Super-

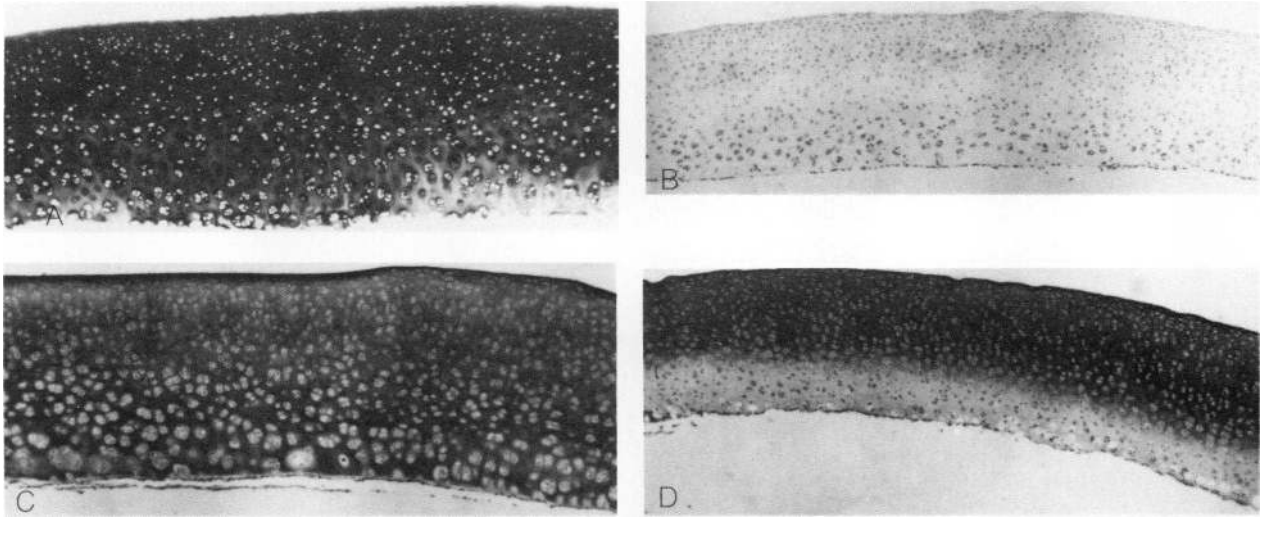

FIGURE 5 Matrix depletion in articular cartilage explants cultured for 12 d assessed by toluidine blue stain for acidic glycosaminoglycans and van Gieson stain for collagen. (A) Toluidine blue. Cartilage explant incubated with culture medium (±). (B) Toluidine blue. Cartilage explant cultured with PHA-stimulated BMC supernate at 20% concentration (++++). Notice the complete loss of metachromasia and the decrease in explant thickness. (C) Van Gieson. Cartilage explant incubated with culture medium (−). (D) Van Gieson. Cartilage explant incubated with PHA-stimulated BMC supernate at 20% concentration (++). Notice the decrease in stainable collagen and explant thickness. Semiquantitative assessment of matrix depletion shown in parentheses. All microphotographs were magnified ×50.

TABLE III

Proteoglycan Depletion in Nasal Cartilage Disks Exposed to Concanavalin A- and Pokeweed Mitogen-stimulated Cell-free Supernates

Supernate	Chondroitin sulfate release	
	Day 4	Day 8
	%±SEM	
Control	12.5±0.7	23.6±1.9
Concanavalin A	20.8±1.6	69.5±4.6
Control	18.8±1.2	45.1±5.5
Pokeweed mitogen	63.1±6.5	87.6±4.6

Mononuclear cell supernates were used at 10% concentration. Chondroitin sulfate released from quintuplicate nasal cartilate disks cultured with medium alone was 10.7±1.1% on day 4 and 20.7±1.9% on day 8 of culture.

nates obtained from mitogen-stimulated BMC induced maximum proteoglycan release from nasal cartilage disks, compared with unstimulated cells. Similar supernates obtained from NAC failed to show PHA-dependent enhancement of MDA release in two separate experiments.

The possibility that adherent cells may be responsible for MDA production was confirmed by experiments with supernates obtained from sheep erythrocyte rosette-purified T lymphocytes. (Table VII). T lymphocytes failed to show a significant increase in MDA production in spite of their ability to mount a virtually intact proliferative response to PHA, as evidenced by incorporation of [^{3}H]thymidine in the responding cells. Moreover, adherent cells separated from subpopulations devoid of T lymphocytes and

TABLE IV

Time of Appearance of Cartilage-degrading Activity in Mononuclear Cell Culture Supernates

Supernate	Time	Chondroitin sulfate release	
		Day 4	Day 8
	h	%±SEM	
Control	24	13.3±1.1	37.3±5.9
	48	15.0±1.0	51.9±8.1
	72	14.3±1.2	46.8±7.1
PHA	24	37.8±9.6	79.5±5.4
	48	29.2±4.2	81.2±1.0
	72	36.1±4.5	81.9±2.6

Mononuclear cell culture supernates harvested at 24, 48, and 72 h were used at 10% concentration. Chondroitin sulfate release from quintuplicate nasal cartilage disks cultured with medium alone was 11.0±0.8% on day 4 and 20.6±1.4% on day 8.

TABLE V

Time of Release of Cartilage-degrading Activity by BMC

Culture period	MDA
h	U*
0–4	<5.0
4–8	14.9
8–16	7.7
16–24	<5.0
24–48	5.9
0–48	15.9

PHA-stimulated BMC supernates were obtained by changing the culture medium from triplicate culture tubes at 4, 8, 16, 24, and 48 h, or from culture tubes left undisturbed for 48 h. MDA was quantitated by assaying the supernates at 5, 10, and 20% concentrations.

* Arbitrary MDA units were calculated as the inverse of the concentration yielding 50% proteoglycan release.

containing >80% phagocytic cells showed spontaneous release of MDA and a failure to respond to PHA in two separate experiments (Table VIII). Recovery of the PHA-dependent increase in MDA production was achieved in reconstituted cell cultures containing both T lymphocytes and adherent phagocytic cells in proportions similar to the original BMC populations. These experiments suggest that MDA may be released by adherent mononuclear cells, presumably monocytes, and that the PHA-dependent increase in MDA release may be T lymphocyte dependent.

TABLE VI

Effect of Removal of Adherent Phagocytic Cells on the Release of Cartilage MDA by BMC

Population	Phagocytic cells	PHA	Chondroitin sulfate release	
			Day 4	Day 8
	%		%±SEM	
Experiment 1				
Medium	—	−	11.0±0.8	20.6±1.4
BMC	15.8	−	11.9±0.7	29.2±3.2
BMC		+	33.2±3.2	78.7±1.9
NAC	0.8	−	17.7±3.4	36.2±7.0
NAC		+	15.0±1.7	32.1±5.0
Experiment 2				
Medium	—	−	19.7±3.4	33.6±4.6
BMC	17.3	−	22.4±4.9	46.5±6.7
BMC		+	43.8±9.0	77.5±5.8
NAC	1.0	−	17.9±1.2	36.0±4.3
NAC		+	20.3±2.3	34.5±4.5

BMC and NAC cultured at a concentration of 2 × 10^6 cells/ml. NAC were obtained after two incubation steps of BMC on plastic petri dishes. Supernates tested in quintuplicate disks for MDA at 10% concentration.

TABLE VII
Release of Cartilage MDA by Partially Purified Mononuclear Cells Populations

Population	Phagocytic cells	PHA	[³H]Thymidine incorporation	Chondroitin sulfate release	
				Day 4	Day 8
	%		*cpm/10⁶ cells*	%±SEM	
BMC	21.2	−	1,882	11.6±0.9	26.6±3.3
BMC		+	109,878	21.5±4.5	73.7±8.0
T	2.0	−	1,373	10.9±0.9	30.5±5.4
T		+	107,460	13.9±2.7	32.2±2.5

BMC and partially purified subpopulations cultured at a concentration of 2×10^6 cells/ml. T lymphocytes (T) were purified by sheep erythrocyte rosetting. All supernates were used at 10% concentration for assay on quintuplicate nasal cartilage disks. [³H]Thymidine incorporation values represent the mean cpm per 10^6 cells of duplicate cultures.

Partial characterization of MDA was achieved by gel chromatography on Ultrogel AcA 44 columns (Fig. 6). When the fractions were tested at 10% concentration, most of the MDA was recovered in the pooled fraction (fraction V) corresponding to an apparent molecular weight ranging from 12,000 to 20,000. Partially purified MDA was also shown to be mediated by live chondrocytes in that frozen and thawed cartilage explants failed to show matrix depletion. This fraction was also able to stimulate collagenase secretion from the cartilage explants (data not shown). MDA in this partially purified fraction was resistant to several cycles of freezing and thawing, but its activity was destroyed by heating at 70°C for 10 min (data not shown).

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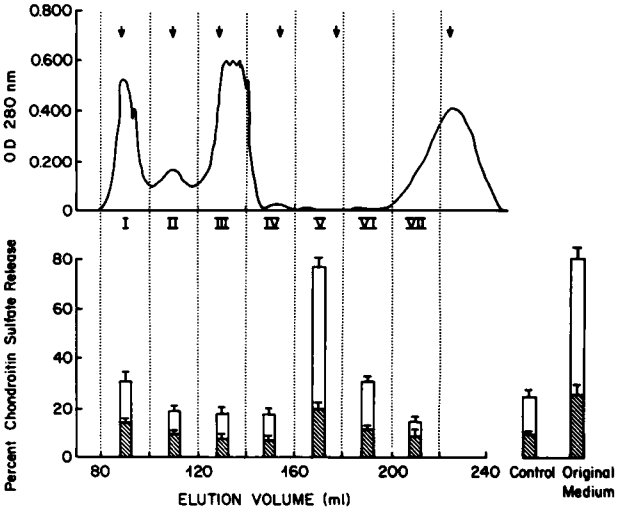

FIGURE 6 Partial characterization by gel column chromatography (Ultrogel AcA44) of cartilage degrading activity in PHA-stimulated BMC supernates. Top: Elution profile of fetal calf serum proteins contained in the supernate. The arrows correspond to the following molecular weight markers, from left to right: void volume (blue dextran), IgG (155,000 mol wt), bovine serum albumin (67,000 mol wt), chymotrypsinogen A (25,000 mol wt), ribonuclease (13,700 mol wt), and phenol red (354 mol wt). Bottom: Matrix-degrading activity in the seven pooled fractions (vertical dotted lines) incubated with nasal cartilage explants at 10% concentration. The active fraction (V) corresponds to an apparent mol wt of 12,000–20,000. At right are shown the activities of culture medium and original active supernate. Hatched areas correspond to the proteoglycan release on day 4.

TABLE VIII
Reconstitution of PHA-dependent Increase in the Production of MDA by Adherent Mononuclear Cells

Population	Phagocytic cells	PHA	Chondroitin sulfate release	
			Day 4	Day 8
	%		%±SEM	
BMC	11.6	−	18.1±1.3	38.8±5.1
BMC		+	25.1±2.2	72.5±4.6
T	1.3	−	18.1±1.0	29.4±1.4
T		+	21.2±3.8	41.6±3.8
M	81.5	−	30.3±5.2	63.0±5.9
M		+	17.3±0.9	48.2±5.2
T + M	21.0	−	17.8±1.7	37.7±6.5
T + M		+	23.2±4.2	70.1±5.6

Results represent mean values of two separate experiments. BMC and T lymphocytes (T) cultured at a concentration of 2×10^6 cells/ml. Monocytes (M) cultured at a concentration of 0.5×10^6/ml. Reconstituted populations (T + M) cultured in a 3:1 ratio at a concentration of 2×10^6/ml. All supernates were used at 10% concentration for assay on quintuplicate nasal cartilage disks. Chondroitin sulfate release for disks cultured with medium alone averaged 16.8±1.2% on day 4 and 29.1±3.4% on day 8.

TABLE IX
Effect of Hydrocortisone Succinate on Nasal Cartilage MDA by Culture Supernates

Supernate	Hydrocortisone	Chondroitin sulfate release	
		Day 4	Day 8
	μg/ml	%±SEM	
Control	—	18.1±4.1	23.2±1.6
	0.1	10.1±0.9	16.7±1.0
	1.0	11.8±1.8	19.4±2.7
	10.0	11.6±1.2	18.4±1.7
PHA-stimulated	—	46.6±7.4	84.1±5.9
	0.1	14.4±1.3	20.9±3.3
	1.0	13.8±1.0	26.5±2.5
	10.0	14.1±0.9	26.6±2.0

factors on protease secretion by connective tissue as well as other cells are inhibited by glucocorticoids, we tested the effects of hydrocortisone succinate on the induction of matrix depletion by active culture supernates (Table IX). Concentrations of the corticosteroid as low as 0.1 μg/ml inhibited MDA to near control levels, and the base-line depletion observed in control cultures was also consistently decreased but to a smaller extent.

DISCUSSION

These data demonstrate that human BMC release one or more factors that stimulate living chondrocytes in organ culture to degrade matrix macromolecules. Matrix degradation was not due to the presence of proteases secreted by monocytes (12, 19–21), because degradation did not take place when the supernates were incubated with dead cartilage explants. Both unstimulated and mitogen-stimulated BMC released MDA, the supernates obtained from stimulated cultures consistently showed higher activity.

Partial purification of stimulated supernates by molecular-sieve chromatography demonstrated most of the MDA in one fraction corresponding to 12,000–20,000 daltons. Although it is possible that MDA is mediated by one molecular species, we cannot rule out the possibility that the crude supernates may contain more than one active factor because we have only used supernates from mitogen-stimulated cells in these purification experiments, and we have only tested the fractions at one concentration. Korn et al. (22), in fact, have shown suppression of fibroblast growth by more than one factor derived from mitogen-stimulated BMC. Moreover, suppression of proteoglycan synthesis by factors present in the crude supernates (23) could increase the fraction of proteoglycan released into the medium by decreasing the total proteoglycan in the explants available for degradation. Although we have not tested the partially purified fraction for its activity to induce collagen degradation, this fraction stimulated secretion of collagenase by cartilage explant chrondrocytes.[2]

In the preliminary experiments described here, MDA appeared to be released by adherent mononuclear cells, presumably monocytes. This suggestion was supported by the experiments in which NAC, depleted by ≥90% of the original number of mononuclear phagocytes failed to show significant MDA, whether stimulated with PHA or not. Moreover, the supernates derived from T lymphocytes partially purified by sheep erythrocyte rosetting did not show a significant increase in MDA when tested at 10% concentration in the face of an intact proliferative response to PHA. The monocyte-enriched population containing >80% adherent phagocytic cells showed variable spontaneous release of MDA and no significant increase in MDA production upon stimulation with PHA. PHA-dependent response, however, was fully restored by the addition of purified T lymphocytes. The high MDA released by unstimulated purified monocyte supernates was observed frequently in our experiments. It is possible that nonspecific activation by fetal calf serum, endotoxin contamination, latex particle phagocytosis, and cell spreading may account for this finding. The apparent decrease of MDA release upon stimulation with PHA is similar to the observations reported by Dayer et al. (24). These authors observed a decrease in collagenase-stimulating factor in supernates obtained from purified monocyte cultures stimulated by PHA or pokeweed mitogen. The significance of these findings is unknown at the present time. The small but variable MDA shown by supernates obtained from purified T cells may be due to the difficulty in totally excluding monocytes from these populations. Lowest MDA has been obtained from T cells containing <0.5% phagocytic cells purified by incubation of BMC on petri dishes before rosetting with sheep erythrocytes (unpublished observations). These preliminary results are only suggestive of the mononuclear phagocyte origin of MDA and of the possible T lymphocyte-mediated enhancement of its release. Further experiments using supernates from purified populations of mononuclear phagocytes stimulated by lymphokine-rich supernates, bacterial lipopolysaccharide, or immune complexes should resolve this issue.

Factors secreted by mononuclear cells have been previously shown to stimulate connective tissue cells, hereby inducing the release of neutral proteases, collagenase, and prostaglandins. Deshmukh-Phadke et al. (7, 8) described a 13,000–15,000-dalton factor derived from activated rabbit macrophages that stimulated the release of collagenase and neutral proteases from isolated chondrocytes. Recently, Ridge et al. (9) were able to confirm these findings by showing that a rabbit macrophage-derived factor stimulated both isolated chondrocytes and cartilage explants to release collagenase and neutral protease. These enzymes were released in the latent form, however, and activation required previous treatment with trypsin or plasmin. Similar findings have been reported by Dayer et al. (10), employing rheumatoid adherent synovial cells as the responding cell. These investigators have shown that the factor produced by mononuclear phagocytes increased the release of latent collagenase and prostaglandins by the synovial cells. The release of this

[2] U. Trechsel, H. E. Jasin, and J. J. Reynolds. Unpublished observations.

14,000-dalton factor was enhanced after stimulation of monocytes by immune complexes, lipopolysaccharides, and lymphocyte secretion products (10, 24). A similar monocyte product was shown by Korn et al. (22) to inhibit fibroblast growth and to stimulate endogenous prostaglandin production. Thus, there is a large body of evidence suggesting that these factors are powerful modulators of connective tissue cell activity. Their role in cartilage destruction, however, has remained unclear, since in most circumstances the enzymes released by the stimulated connective tissue cells are found mostly in the latent form (3, 7–10), or are complexed to protease inhibitors (2, 3, 25, 26). We present here the first direct evidence that factors produced by human mononuclear cells are able to induce degradation of intact living cartilage. Our data show that the factor responsible for MDA may have the similar molecular size, cellular origin, time of appearance in culture, and heat sensitivity as those described by Dayer et al. (10), Deshmukh-Phadke et al. (7, 8), and Korn et al. (22). In addition, our active supernates are also able to stimulate release of collagenase by live cartilage explants.[3] This evidence is compatible with the possibility that we may be dealing here with similar factors described previously.

Recent evidence (27) suggests that one of the human monocyte factors (10) that stimulate connective tissue cells may be similar or identical to the monokyne identified as lymphocyte-activating factor or interleukin 1 (28), which modulates various facets of T and B lymphocyte activation and differentiation. Another property shared by lymphocyte-activating factor is suggested by our data showing that MDA is exquisitely inhibited at the level of the chondrocyte by low concentrations of corticosteroids. Recent work by Smith et al. (29) has shown that adrenal steroids also suppress lymphocyte-activating factor activity by inhibiting the secretory response of the target cell, the T lymphocyte.

A different source of factors capable of cartilage matrix degradation through stimulation of chondrocytes has been described by the Strangeways Laboratory group (4, 5). A 20,000-dalton factor secreted by normal porcine synovial tissue has been shown to mediate cartilage matrix depletion in live explants. Further identification of these tissue factors and their relationship to the factors released by mononuclear cells will be possible when more precise chemical characterization is available. Nevertheless, it is clear that the functional consequences of the interaction of these peptide messengers with the chondrocytes may be similar.

It is likely that cartilage destruction in human inflammatory and degenerative joint diseases is the result of a complex process involving many different cellular and enzymatic mechanisms. Although collagenase and proteases found in inflammatory synovial fluids (3) have been held responsible for matrix macromolecule degradation (1–3), their importance in cartilage destruction in rheumatoid arthritis is open to question since much of the irreversible cartilage destruction in this disease is mediated by the invading pannus (30–32). Another but not exclusive mechanism supported by previous studies (4, 6) would implicate the enzymes of activated chondrocytes and pannus cells, or their secretion products exerting cartilage-degrading activity locally. There is abundant evidence that the resorptive activity mediated by synovium and chondrocytes takes place only in close proximity to the cell surface. Fell and Jubb (4) showed that normal rabbit synovial tissue co-cultured in contact with dead cartilage explants was still able to induce degradation, whereas live chondrocytes were necessary when the tissue was co-cultured in the same dish but not in contact with the cartilage explant. Steinberg et al. (6) showed that rheumatoid synovium co-cultured with bovine nasal cartilage explants increased matrix proteoglycan degradation. These authors confirmed the observation that live chondrocytes were required for optimal proteoglycan degradation. The work described here highlights the possible role of mononuclear cell infiltrates in cartilage resorption.

In cartilage explants undergoing degradation mediated by synovial cell factors, the areas containing dead chondrocytes are usually spared from proteoglycan depletion (5). Moreover, Dingle and Dingle (33) have shown that matrix degrading activity takes place only in the pericellular area in cartilage explants, very close to the chondrocyte cell membrane. Mammalian collagenases are released in a latent form, either as zymogens (34, 35) or as enzyme-inhibitor complexes (36, 37). In either case, enzyme activation takes place after cleavage by proteolytic enzymes such as trypsin or plasmin (2, 38). It is possible that proteases either membrane bound (39) or secreted by chondrocytes and pannus cells could activate collagenase in the pericellular space. The active collagenase would then be able to exert its action locally, before the formation of inactive enzyme-inhibtor complexes.

Factors secreted by synovial cells and tissue macrophages may play a role in cartilage degradation in degenerative joint diseases (5, 7, 8). In chronic inflammatory joint diseases such as rheumatoid arthritis, interactions between lymphoid cells engaged in active local immune responses take place in the involved synovial tissue (40, 41) with production of peptide messengers (42) similar to the mononuclear cell products described here. Our data suggest that chondro-

[3] U. Trechshel, H. E. Jasin, and J. J. Reynolds. Unpublished observations.

cyte stimulation mediated by such factors may play a role in cartilage resorption, although their importance in vivo has not been established. Further investigation of the presence of mononuclear cell factors in pathologic synovial fluids and identification of their cellular origin may demonstrate in detail the importance of this process in irreversible cartilage damage in human disease.

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