EFFECT OF A MONONUCLEAR CELL CULTURE MEDIUM ON COLLAGEN AND GLYCOSAMINOGLYCAN PRODUCTION BY SYNOVIAL CELLS IN CULTURE

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1. Introduction

The regulation of connective tissue metabolism involves cell–cell interactions leading to modulation in the enzymic breakdown of collagen and proteoglycans as well as in the synthesis rate of these macromolecules. In many chronic inflammatory lesions, such as rheumatoid synovial pannus, numerous cell types (lymphocytes, monocytes, fibroblasts, endothelial cells) are closely associated. A substance produced by an immune cell may influence the main functions of fibroblasts: proliferation, secretion of degradative enzymes (collagenase, proteoglycan-degrading proteases), and synthesis of collagen and proteoglycans. Experimental granuloma, tissue and cell culture have provided tools to detect a growing number of such factors [1–9].

A mononuclear cell factor that stimulates collagenase and prostaglandin production by rheumatoid synovial cells in culture has been isolated [10]. This work reports the inhibition of collagen and non-collagenous protein synthesis by synovial cells, induced by a factor(s) of mononuclear cell medium (MCM). This factor(s), in addition, significantly raises the level of glycosaminoglycan (GAG) production. Such biologically active monocyte-derived substance(s) might be involved in the connective tissue metabolism of inflammatory lesions.

2. Materials and methods

2.1. *Culture of synovial cells*

Synovial cells were obtained by enzymatic dissociation from osteoarthritic surgical specimens as in [10], and maintained in DMEM, supplemented with 10% foetal calf serum (FCS), 2 mM glutamine, penicillin (100 units/ml), streptomycin (100 μg/ml) and Fungizone (2.5 μg/ml). Experiments were performed on cells at first passage.

2.2. *Mononuclear cell medium (MCM)*

Mononuclear cells from blood of healthy donors were separated by centrifugation in a Ficoll Metrizoate solution and incubated in McCoy medium, with phytohaegglutinin (40 μg/ml) as in [11]. After 3 days, the culture medium was collected, centrifuged and the supernatant stored at −25°C.

2.3. *Incubation of synovial cells with MCM*

Synovial cells were plated at 2.5×10^5 cells/Petri dish (6 cm) and grown to confluency. The medium was changed 24 h before experiment and the supernatant was added to the fresh medium at final dilution of 1:10 or 1:15.

2.4. *Collagen assay*

Cells were first incubated with 1.5 ml medium containing β-aminopropionitrile (50 μg/ml) and ascorbic acid (10 μg/ml), with or without MCM. After 24 h, the medium was again replaced with medium containing the same additions plus 2.5–5 μCi/ml L-[4-5-^{3}H]proline (20–30 Ci/mmol, CEA). At the end of the incubation, media and cells were precipitated separately with 5% trichloroacetic acid. Then the pellets were submitted to purified bacterial collagenase treatment as in [12] and the radioactivity of collagen and noncollagen proteins was determined.

2.5. *Glycosaminoglycan assay*

Confluent cultures were incubated in DMEM, 10%

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FCS in presence of [1-^{3}H]glucosamine (2.5 μCi/ml); spec. act. 11 Ci/mmol) for 78 h. Radioactive GAG present in the culture medium (extracellular pool), in the supernatant obtained after trypsinisation of the cells (pericellular pool) [13] and in the cell pellet (intracellular pool) were prepared as in [11]. The radioactivities of these fractions were assayed by liquid scintillation counting after dissolution in distilled water.

3. Results

3.1. *Kinetics of collagen production*

Exposure of synovial cells to a 1:10 dilution of MCM resulted in a dramatic decrease in the production of [^{3}H]proline-labeled protein, which was susceptible to digestion by collagenase (fig.1a). This inhibitory effect could be detected after only 6 h incubation and, by 48 h, a 90% inhibition of collagen production in the medium was observed. Cell-associated collagen exibited a similar decrease which began later, between 12–24 h exposure (fig.1b). The small difference between control and treated cells observed prior 12 h was not significant. When expressed as % of total collagen radioactivity, the cell content of exposed cultures displayed a profile similar to controls (fig.1c). This demonstrates that there was no accumulation of collagen inside the cells and that the secretion of this protein was not affected. The decrease in collagen production cannot be accounted for by a decrease in cell number since experiments were done at cell confluency, with a fairly constant cell density as shown by enumerations made at the end of treatment. Also practically excluded was the inhibitory effect of prostaglandin PGE_2 on the proliferation rate of synovial cells, occuring after exposure to MCM [10]. In addition, the viability of cells was not affected in our experiments, as judged by the trypan blue exclusion test.

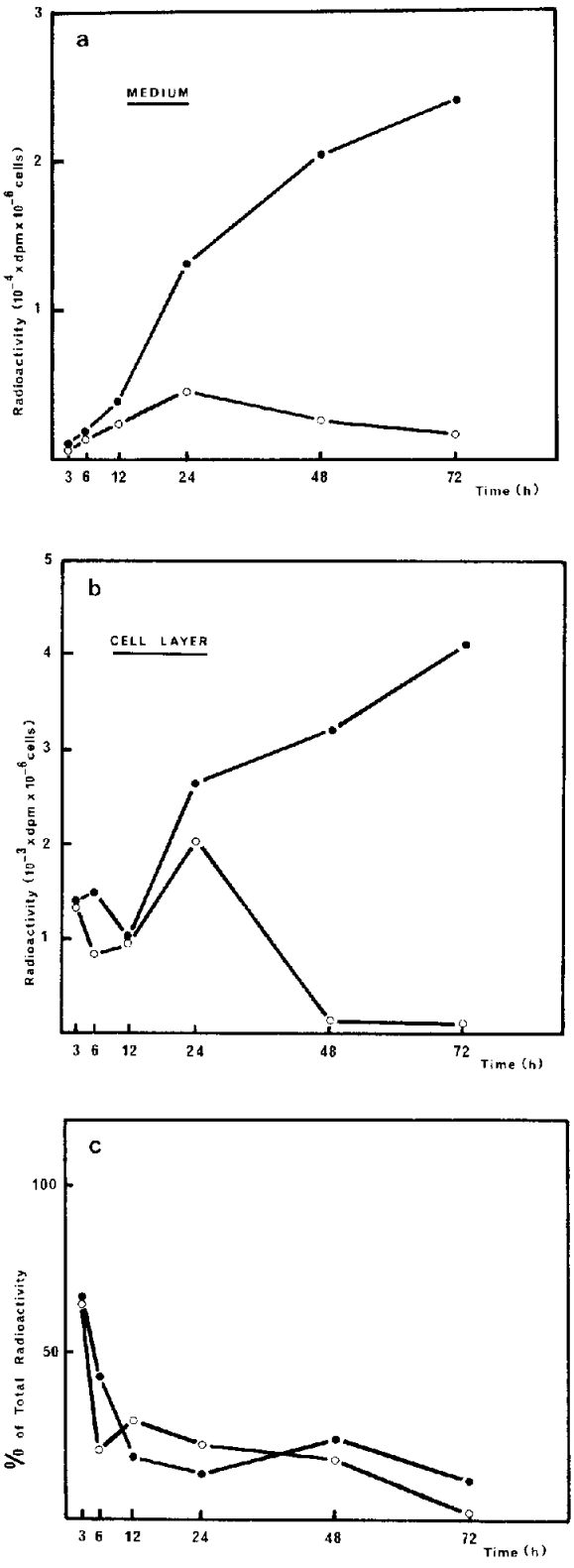
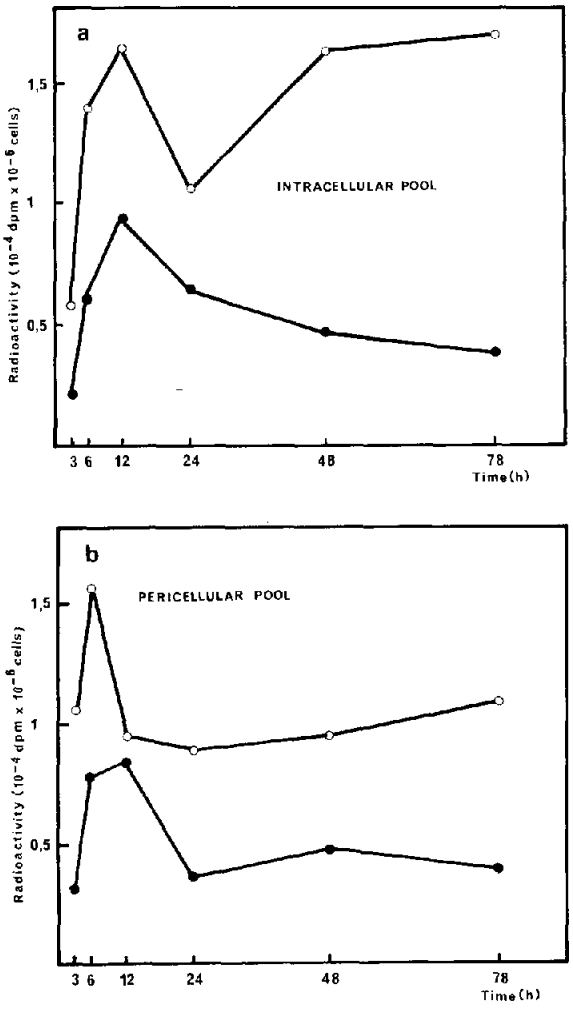


Fig.1. Effect of MCM on collagen synthesis by synovial cells in culture: (a) Collagen production in the culture medium; incubation with 2.5 μCi/ml [^{3}H]proline; data represent mean of duplicate cultures; (b) collagen content of the cell layer; (c) collagen of the cell layer expressed as % of total collagen; control cultures (–•–); MCM-treated cultures (–○–).

Table 1
Effect of two different preparations of MCM (MCM_1 and MCM_2) on synovial cell collagen and non-collagen protein production

	Collagen		Non-collagen protein	
	dpm × 10^{-6} cells	%	dpm × 10^{-6} cells	%
Control	87 290	100	116 043	100
MCM_1 1:15	61 827	70.8	115 102	99.2
MCM_1 1:10	59 741	68.4	111 214	95.8
Control	87 290	100	116 043	100
MCM_2 1:15	51 016	58.4	88 695	76.4
MCM_2 1:10	41 220	47.2	76 404	65.8

Cultures were exposed to 1:10 and 1:15 dilutions of MCM and labeled with 5 μCi/ml [^{3}H]proline during the last 48 h. Medium was assayed for collagen and non-collagen protein. Data represent mean of duplicate cultures



3.2. *Non-collagen proteins*

The decrease in protein synthesis was not specific to collagen since production of non-collagenase-sensitive proteins was also reduced, though to a lower extent. The effect of MCM on non-collagen protein varied with the MCM preparation and the dilution used. As shown in table 1, some preparations which produced little effect on non-collagen protein production could still exert a significant decrease on collagen synthesis (~30%). Thus, collagen was more sensitive to MCM than non-collagen protein synthesis.

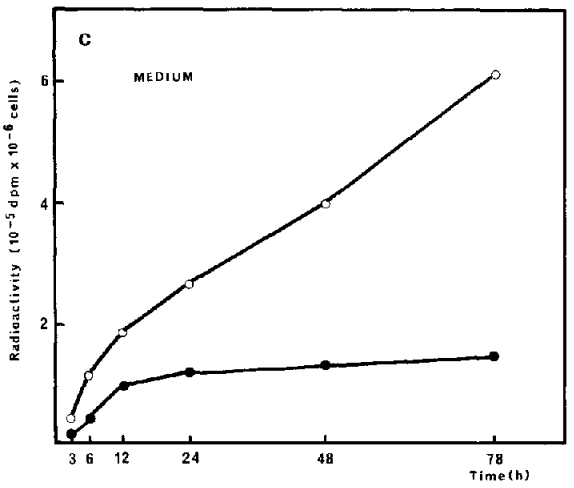


Fig.2. Effect of MCM on GAG synthesis by synovial cells in culture. (a) Intracellular pool; incubation with 2.5 μCi/ml [^{3}H]glucosamine; data represent mean of duplicate cultures; (b) pericellular pool; (c) culture medium; control cultures (–●–); MCM-treated cultures (–○–).

3.3. *Glycosaminoglycans*

Synovial cells exposed to MCM synthesized increased GAG levels since the radioactivity of cellular (fig.2a) and pericellular (fig.2b) pools from treated cultures were 2–2.5-times higher than in the controls. GAG production in the medium of the control cultures increased within 24 h and then remained relatively constant. In contrast, cells exposed to MCM secreted increasing amount of radioactive GAG up to 78 h, at which time the level of newly synthesized GAG present in the medium was 4-times greater than the control (fig.2c). However, the relative distribution of labeled GAG in the different pools was not affected by MCM (fig.3).

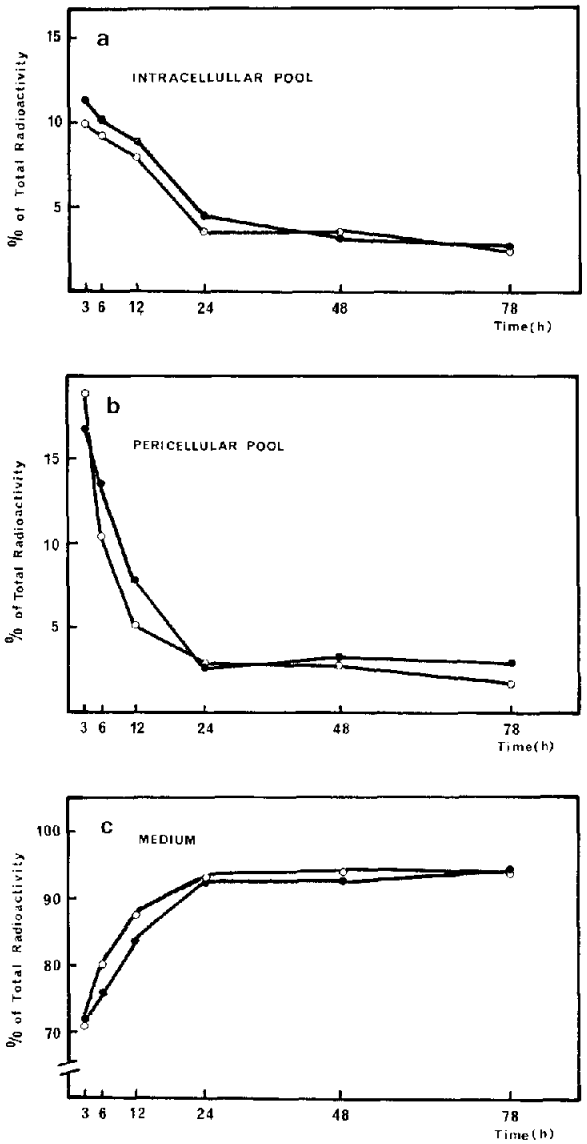


Fig.3. GAG content of the different fractions expressed as % of total GAG: (a) intracellular pool; (b) pericellular pool; (c) culture medium; control cultures (•); MCM-treated cultures (–○–).

4. Discussion

This work shows that stimulated peripheral blood monocytes contain a factor(s) which affects synovial cells in culture, depressing markedly collagen synthesis and other protein synthesis, though to a smaller extent, while enhancing GAG production. MCM has also been shown to decrease collagen neosynthesis in skin fibroblasts [14], without affecting non-collagen protein production. This difference might be related to the MCM preparation used as shown in table 1. The decrease in collagen content of MCM-treated cultures cannot be explained by extracellular enzymic degradation since collagenase secreted into the medium was inhibited by serum and has to be demonstrated by previous proteolytic activation [10,11].

MCM enhances hyaluronic acid secretion by synovial cells in culture [11]: this work extends that finding to the totality of the GAG produced by these cells.

A mononuclear cell factor capable of increasing collagenase and prostaglandin PGE_2 release by synovial cells has been isolated [15]. We do not know whether the same molecule is also involved in the modulation of collagen and GAG synthesis observed in our work. Further investigations, in progress, are needed to clarify this point.

PGE_2 is known to be a relatively specific inhibitor of collagen synthesis by osteoblasts [16]. It can also increase hyaluronic acid production by synovial fibroblasts [17,18] and GAG biosynthesis in skin fibroblasts [19]. So, the effects observed in our experiments could be related to the secondary influence of a rise in PGE_2 release by MCM-treated synovial cells [10]. They cannot be due to contaminating amounts of PGE_2 already present in monocyte-conditioned medium since the production of PGE_2 by monocytes is insignificant compared to that produced by the synovial cells exposed to MCM [20].

Although the inflamed synovial tissue is infiltrated with monocytes, the relevance of these findings to the pathogenesis of rheumatoid arthritis is not clear since they do not completely integrate the fibroblastic

response occurring during chronic inflammation. They only provide some explanation for a part of the events which modulate collagen and GAG production. For example, the present experiments could not explain the increased collagen content observed in rheumatoid synovial tissue as compared to normal tissue [21–23]. However, other cell-types present in inflammation can produce substances which stimulate collagen synthesis in fibroblasts, i.e., counteracting the action of MCM. Such factors have been reported in lymphocytes [5], macrophages [6] and platelets [8]. Furthermore, it has been shown that connective tissue activating factors can be detected in the proliferation phase of the inflammation process whereas inhibitory substances appear as the disease progresses [24,25]. These studies suggest, therefore, that there is normally an adequate balance between stimulation and inhibition, but which may be perturbed under certain conditions resulting in either deficient or excessive accumulation of connective tissue macromolecules. The monocyte derived factor may be involved in this process as one of these factors.

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References

[1] Heppleston, A. G. and Styles, J. A. (1967) Nature 214, 521–522.
[2] Castor, C. W. (1975) Ann. NY Acad. Sci. 256, 304–317.
[3] Johnson, R. L. and Ziff, M. (1976) J. Clin. Invest. 58, 240–252.
[4] Randoux, A. J., Maquart, J., Cornillet-Stoupy, G., Szymanowicz, G. and Borel, J. P. (1978) 6th Coll. Eur. Fed. Conn. Tissue Clubs, Paris, 257–258.
[5] Wahl, S. M., Wahl, L. M. and McCarthy, J. B. (1978) J. Immunol. 121, 942–946.
[6] Aalto, M., Turakainen, H. and Kulonen, E. (1979) Scand. J. Clin. Lab. Invest. 39, 205–213.
[7] Jimenez, S. A., McArthur, W. and Rosenbloom, J. (1979) J. Exp. Med. 150, 1421–1431.
[8] Bańkowski, E., Nowak, H. F. and Jodczyk, K. J. (1980) Exp. Pathol. 18, 462–467.
[9] Vaes, G. (1980) Agents and Actions 10, 474–485.
[10] Dayer, J. M., Krane, S. M., Russell, R. G. G. and Robinson, R. D. (1976) Proc. Natl. Acad. Sci. USA 73, 945–949.
[11] Bocquet, J., Heron, J. F., Pujol, J. P., Belierd, R., Bonaventure, J. and Loyau, G. (1980) Int. J. Tiss. Reac. 2, 127–136.
[12] Peterkofsky, B. and Diegelman, R. (1971) Biochemistry 10, 988–993.
[13] Gamse, G., Fromme, H. G. and Kresse, M. (1978) Biochim. Biophys. Acta 544, 514–528.
[14] Jimenez, S. A., McArthur, W. and Rosenbloom, J. (1979) J. Exp. Med. 150, 1421–1431.
[15] Dayer, J. M., Stephenson, M. L., Schmidt, E., Karge, W. and Krane, S. M. (1981) FEBS Lett. 124, 253–256.
[16] Raisz, L. G., Koolemans-Beynen, A. R. (1974) Prostaglandins 8, 377–385.
[17] Castor, C. W. (1975) J. Lab. Clin. Med. 85, 392–404.
[18] Yaron, M., Yaron, I., Wiletski, C. and Zor, U. (1978) Arthritis Rheum. 21, 694–698.
[19] Schonhofer, P. S., Peters, H. D., Wasmus, A., Peskar, B. A., Von Figura, K. and Klappstein, I. (1978) Pol. J. Pharmacol. Pharm. 30, 183–193.
[20] Dayer, J. M., Breard, J., Chess, L. and Krane, S. K. (1979) J. Clin. Invest. 64, 1386–1392.
[21] Vitto, J., Lindy, S., Turto, H. and Vainio, K. (1972) J. Lab. Clin. Med. 79, 960–971.
[22] Weiss, J., Leibovich, S. J., Hunter, J. A. A. and Cawley, M. I. D. (1974) Ann. Rheum. Dis. 33, 404.
[23] Lovell, C. R., Nicholls, A. C., Jayson, M. I. V. and Bailey, A. J. (1978) Clin. Sci. Mol. Med. 55, 31–40.
[24] Kulonen, E. and Potila, M. (1980) Exp. Path. 18, 456–461.
[25] Neilson, E. G., Jimenez, S. A. and Phillips, M. (1980) J. Immunol. 125, 1708–1714.