Biochem. J. (1985) **230**, 83–88
Printed in Great Britain

Mast-cell products and heparin stimulate the production of mononuclear-cell factor by cultured human monocyte/macrophages

Joan R. YOFFE, David J. TAYLOR and David E. WOOLLEY
University Department of Medicine, University Hospital of South Manchester, West Didsbury, Manchester M20 8LR, U.K.

(*Received 11 January 1985/18 April 1985; accepted 8 May 1985*)

Purified mast cells derived from rat peritoneal fluids and dog mastocytomas were extracted with 1 M-NaCl and sonication techniques. The mast-cell products increased the production of mononuclear cell factor from human peripheral blood mononuclear cells in culture, as judged by the enhanced stimulation of prostaglandin E (2–5-fold) and collagenase (3–11-fold) production by cultured adherent synovial cells. Heparin alone (1–10 μg/ml) induced a similar stimulation of mononuclear-cell-factor production by monocyte cultures, whereas histamine (1–10 μg/ml) had no effect. The stimulatory effect of mast-cell products and heparin represented a direct effect on mononuclear cells; they did not potentiate the effect of monokine on the synovial cells. These results suggest that mast-cell–macrophage interactions may play a significant role in the pathogenesis of inflammation and connective-tissue degradation.

Mast cells are widely distributed in connective tissues, and increased numbers have been reported in many inflammatory disorders. They are found in increased numbers in rheumatoid synovial tissue (Janes & McDonald, 1948; Crisp *et al.*, 1984; Godfrey *et al.*, 1984; Bromley & Woolley, 1984), but despite suggestions that mast cells may be important in the pathogenesis of connective-tissue disease, there is little information on their role in the pathophysiology of joint destruction in rheumatoid arthritis.

Rheumatoid synovial tissue has the capacity to produce several cartilage-degrading enzymes, especially collagenase and elastase, which have been localized at sites of cartilage erosion (Woolley *et al.*, 1977; Menninger *et al.*, 1980). Cultured adherent synovial cells (ASC) with fibroblastic and dendritic morphology were responsible for most of the collagenase production *in vitro* (Woolley *et al.*, 1979). Moreover, the stimulation of collagenase production by these cells resulted from complex cellular interactions with macrophages and T-lymphocytes (Dayer *et al.*, 1980; Krane, 1981). Macrophages are known to stimulate cultured ASC to produce increased amounts of prostaglandins and collagenase by the production of a monokine termed 'mononuclear-cell factor' (MCF), which is akin to interleukin-1 (Mizel *et al.*, 1981). Recent histochemical studies have demonstrated both mast cells and macrophages at sites of cartilage erosion in rheumatoid joints (Bromley *et al.*, 1984). Mast cells have also been shown to contain soluble factors that similarly stimulate cultured ASC to produce PGE and collagenase (Yoffe *et al.*, 1984). In the present study we have used '*in vitro*' techniques to examine the effects of mast-cell products on the production of MCF from cultures of human peripheral blood mononuclear cells. We report here that mast cells have the potential to stimulate mononuclear cells to produce increased amounts of MCF, a cellular interaction that could have profound effects on the mechanism of inflammation, proteolysis and connective-tissue degradation *in vivo*.

Abbreviations used: ASC, adherent synovial cells; MCF, mononuclear-cell factor; DMEM, Dulbecco's modified Eagle's medium; HBSS, Hanks balanced salt solution; FCS, foetal-calf serum; PGE, prostaglandin E; MCP, mast-cell products; MCCM, mononuclear-cell conditioned media.

Methods

Materials

DMEM and HBSS were obtained from Gibco, Paisley, Renfrewshire, Scotland, U.K. Heat-inactivated FCS and Costar plastic tissue-culture multi-dishes were obtained from Northumbria

Table 1. *Comparative analysis of different preparations of MCP*

Mast-cell preparation	(*a*) Mediator content in MCP at 5% (v/v) (equivalent to 5×10^4 mast cells/ml)			(*b*) Relative stimulation (fold) of subcultured ASC by MCP at 10% (v/v)	
	Histamine (μg/ml)	Heparin/glycosaminoglycan (μg/ml)	PGE (ng/ml)	PGE	Collagenase
Rat	1.0	3.2	<0.55	2.6	0
Dog					
A	0.9	3.3	21.0	700	48
B	0.2	1.5	0.2	120	18
C	1.9	12.0	7.5	75	10

Biologicals, Cramlington, Northd., U.K. Histamine acid phosphate, porcine mucosal heparin Grade 1, *o*-phthalaldehyde, pancreas trypsin [tosylphenylalanylchloromethane ('TPCK')-treated], Hypaque (Ficoll/Metrizamide) and concanavalin A were obtained from Sigma, Poole, Dorset, U.K. Soya-bean trypsin inhibitor was obtained from Worthington Biochemicals, Freehold, NJ, U.S.A., and dimethyl Methylene Blue from Uniscience, Cambridge, U.K. [^{3}H]PGE_2 was obtained from Amersham International, Amersham, Bucks., U.K. and PGE antiserum from Miles Laboratories, Slough, Berks., U.K.

Mast-cell products (MCP)

MCP were prepared by sonication and 1 M-NaCl extraction of purified suspensions of rat peritoneal mast cells (>95% purity) or dog mastocytoma mast cells (>90% purity), as described previously (Yoffe *et al.*, 1984). Granular debris was removed by centrifugation at 36000*g* for 45min at 4°C and Millipore filtration. MCP were diluted to the equivalent of 10^6 cells/ml and added to mononuclear-cell cultures at 5% (v/v). Analysis of the preparations used in the present study is shown in Table 1. Histamine and heparin/glycosaminoglycan were assayed with *o*-phthalaldehyde (Shore *et al.*, 1959), and dimethyl Methylene Blue (Farndale *et al.*, 1981) respectively. We have previously shown that whole MCP at 10% (v/v) stimulated both PGE and collagenase production by ASC (Yoffe *et al.*, 1984). The relative stimulating effect of each of the MCP preparations used in the present study on cultures of ASC is shown in Table 1.

Mononuclear-cell cultures

Mononuclear cells were prepared by density-gradient centrifugation of citrated human peripheral blood on Hypaque. Cell suspensions (10^7 cells/ml) in DMEM supplemented with 10% (v/v) FCS were prepared and 1 ml aliquots pipetted into 32mm-diameter six-well plastic culture dishes. Non-adherent cells were removed after 3–4h by washing with HBSS. Triplicate cultures of adherent mononuclear cells (monocyte/macrophages and T-lymphocytes) were incubated at 37°C for 3 days in 1 ml of DMEM/10% FCS containing the substance(s) under investigation in a water-saturated atmosphere of CO_2/air (1:19). Substances studied were rat and dog mast-cell products at 5% (v/v), histamine at 1–10μg/ml, heparin at 1–100μg/ml and concanavalin A at 100μg/ml. MCCM were harvested and stored at −20°C.

ASC cultures

Confluent monolayers of human rheumatoid synovial fibroblasts (second to fourth passage) were prepared as described previously (Dayer *et al.*, 1980). Cells were incubated with 10% (v/v) MCCM in DMEM/10% FCS for 24h. This culture medium was removed and subsequently assayed for PGE. After washing the cells with HBSS they were incubated a further 48h in serum-free DMEM containing 10% (v/v) MCCM. The culture medium was then removed, stored at −20°C, and subsequently assayed for collagenase activity.

Assays

PGE was measured in culture medium by radioimmunoassay (Levine *et al.*, 1971) by utilizing an antiserum with similar specificity towards PGE_1 and PGE_2.

Collagenase activity was assayed after trypsin activation (10μg/ml) for 20min followed by the addition of 50μg of soya-bean trypsin inhibitor/ml using [^{14}C]glycine-labelled collagen (Woolley *et al.*, 1978) in the diffuse-fibril assay (Cawston & Barrett, 1979). One unit of collagenase activity is equivalent to 1μg of [^{14}C]collagen degraded/h per ml of culture medium.

Each experiment was repeated by using three different preparations of mononuclear cells, tested on several adherent rheumatoid synovial-cell cultures. Although all showed qualitative similarities, there were quantitative variations between experi-

Table 2. *Evaluation of bioassay for MCF: measurement of stimulation of PGE and collagenase production by subcultured ASC*

MCCM added	Concn. (%, v/v)	PGE (ng/24h per ml)	Collagenase (units/48h)
None (control)	—	<0.55	<0.1
MCCM containing MCF	5	3.7±1	2.7±2
	10	10.9±2	7.3±2
MCCM derived from cultures containing concanavalin A (100 μg/ml)	1	4.5±5	2.0±2
	5	19.4±4	15.3±2
	10	39.5±4	35.0±3

ments and, for this reason, results from one representative experiment are presented. All test incubations were performed in at least triplicate and data represent mean values ± S.E.M.

Results

The lectin concanavalin A has previously been reported to be one of the most effective stimuli for monokine production (Dayer *et al.*, 1980). The effect of MCF on the production of PGE and collagenase by passaged ASC was shown to be proportional to the concentration of MCCM added. However, at low concentrations of MCCM the stimulation of collagenase production was not as pronounced as that shown by the PGE assay (Table 2). Thus the measurement of PGE and collagenase production by stimulated ASC provides a bioassay for the quantitative assessment of MCF, at least for MCCM concentrations up to 10% (v/v).

Addition of MCCM at 10% (v/v) to passaged ASC induced a marked increase in PGE and collagenase production (Tables 3 and 4), a response that has been ascribed to the action of MCF. MCCM derived from mononuclear cells that had been exposed to either rat or dog A and C MCP in culture produced a much greater increase in MCF, as judged by increased levels of PGE and collagenase from ASC cultures. Tables 3 and 4 show that conditioned media from peripheral-blood mononuclear cells exposed to 5% (v/v) rat or dog A and C MCP brought about a 2–5-fold increase in PGE and a 3–11-fold increase in collagenase production when compared with conditioned medium from untreated mononuclear cells. Moreover both MCP- and concanavalin A-treated MCCM produced comparable responses. The effectiveness of the mast-cell preparations to stimulate the production of MCF was different to their ability to stimulate ASC directly (Table 1*b*), an observation that suggests that different mast-cell-activating factors exist for mononuclear cells and ASC respectively.

As the MCF-stimulating activity (Tables 3 and 4) appear related to the amounts of histamine and heparin in each MCP preparation (Table 1*a*), we examined the effect of treating the mononuclear cells with pure histamine or heparin at concentrations similar to those present in the mast-cell extracts. Conditioned media from mononuclear

Table 3. *Effect of MCP on the production of MCF as measured by PGE production by ASC*

Confluent monolayers of passaged ASC were treated with 10% MCCM in DMEM/10% FCS for 24h. MCCM was prepared from mononuclear cells (control) or from similar cells exposed to 5% MCP or concanavalin A as described in the Methods section. Results are means ± S.E.M. for five determinations. *$P<0.01$ (Student's t test).

Treatment	PGE (ng/24h per ml)
DMEM/FCS No MCCM	<0.55
MCCM control	56.3±4.1
+Rat MCP	99.4±16.3*
+Dog A MCP	114.3±5.8*
+Dog B MCP	29.4±11.8
+Dog C MCP	311.1±57.0*
+Concanavalin A	179.1±44.0*

Table 4. *Effect of MCP on the production of MCF as measured by collagenase production by ASC*

Confluent monolayers of passaged ASC were treated with 10% MCCM in serum-free DMEM for 48h. MCCM was prepared from untreated mononuclear cells (control) or from similar cells exposed to 5% MCP or concanavalin A as described in the Methods section. Results are means ± S.E.M. for five determinations. *$P<0.01$ (Student's t test).

Treatment	Collagenase (units/48h)
DMEM No MCCM	<0.1
MCCM control	9.5±6.0
+Rat MCP	33.7±3.0*
+Dog A MCP	37.4±4.6*
+Dog B MCP	8.0±6.6
+Dog C MCP	105.9±6.7*
+Concanavalin A	36.8±6.4*

Table 5. *Effect of heparin and histamine on the production of MCF as measured by PGE production by ASC*
Confluent monolayers of passaged ASC were treated with 10% MCCM in DMEM/10% FCS for 24h. MCCM was prepared from untreated mononuclear cells (control) or from similar cells exposed to 1 or 10 μg of heparin/ml and 1 or 10 μg of histamine/ml as described in the Methods section. Results are means ± S.E.M. for five determinations. *$P < 0.01$ (Student's t test).

Treatment	PGE (ng/24h per ml)
DMEM/FCS	<0.55
No MCCM	
MCCM control	206.3 ± 25.1
+ Heparin (1 μg/ml)	327.7 ± 14.7*
+ Heparin (10 μg/ml)	411.8 ± 11.8*
+ Histamine (1 μg/ml)	272.8 ± 61.0
+ Histamine (10 μg/ml)	204.8 ± 44.8

Table 6. *Effect of heparin and histamine on the production of MCF as measured by collagenase production by ASC*
Confluent monolayers of passaged ASC were treated with 10% MCCM in serum-free DMEM for 48h. MCCM was prepared from untreated mononuclear cells (control) or from similar cells exposed to 1 or 10 μg of heparin/ml and 1 or 10 μg of histamine/ml as described in the Methods section. Results are means ± S.E.M. for three determinations. *$P < 0.01$ (Student's t test).

Treatment	Collagenase (units/48h)
DMEM	<0.1
No MCCM	
MCCM control	21.0 ± 2.1
+ Heparin (1 μg/ml)	51.2 ± 5.7*
+ Heparin (10 μg/ml)	45.5 ± 6.4*
+ Histamine (1 μg/ml)	29.3 ± 6.0
+ Histamine (10 μg/ml)	20.1 ± 2.0

cells treated with heparin at 1 or 10 μg/ml consistently caused an enhanced activation of ASC that resulted in an approx. 2-fold increase of PGE and collagenase production (Tables 5 and 6). Higher doses of heparin (50–100 μg/ml) on the mononuclear cells had a variable effect. Conditioned medium from mononuclear cells treated with histamine (1 or 10 μg/ml) did not significantly enhance the stimulation of PGE and collagenase production compared with that from untreated mononuclear cells (Tables 5 and 6). The MCF-stimulating factor in dog MCP was subsequently shown to be non-dialysable and stable to boiling for 2min, thereby providing further evidence that mast-cell heparin may be the factor involved in stimulating MCF production.

As MCCM exposed to MCP would contain residual amounts of histamine, heparin and other factors, the effects of equivalent concentrations of these components on PGE and collagenase production by synovial cells was examined. Neither rat and dog MCP at 0.5% (v/v) had any effect on PGE or collagenase production by ASC. Similarly, neither heparin at 1 μg/ml nor histamine at 1 μg/ml had any effect on synovial-cell production of PGE or collagenase. Moreover, when these substances, at the concentration described, were added to ASC cultures in addition to 10% (v/v) control MCCM, no significant increase in prostaglandin or collagenase production was measured. This indicated that the mast-cell products did not potentiate the effect of MCF on the adherent cells, at least at the concentrations used in these experiments.

Discussion

The results presented here suggest that whole MCP can interact with mononuclear cells to stimulate the production of MCF, and that their ability to do so correlates with their histamine and heparin content. The ability of low concentrations of heparin to mimic the stimulation of MCCM induced by *whole* MCP suggests it may be the mast-cell factor involved. However, there is substantial evidence for mast-cell heterogeneity. (Barrett & Metcalfe, 1984), and some mast cells contain oversulphated chondroitin sulphate rather than heparin (Razin *et al.*, 1984), but as yet the action of these other heparin-related polysaccharides on monokine production has not been examined.

The mechanism by which MCP and heparin increase the production of MCF by human monocyte/macrophages is not clear, but there are several reports that mast cells may interact with macrophages to influence the secretion of other factors. Macrophages have been observed to ingest mast-cell granules (Lindahl *et al.*, 1979; Baggiolini *et al.*, 1982) and this has been shown to be a necessary prerequisite for the release of platelet activating factor (Mencia-Huerta & Beneveniste, 1979; Camussi *et al.*, 1980). Mouse macrophages possess heparin receptors on the cell surface (Bleiberg *et al.*, 1983), and it seemed likely that heparin might 'activate' the monocyte/macrophage cultures in the present study. Heparin and other polyanions have been reported to stimulate macrophages (Jaques, 1979), including the release of interferon (Schultz *et al.*, 1977) and the promotion of lysosomal-enzyme secretion (Schorlemmer *et al.*, 1977). Interestingly, the stimulation of interferon in relation to the concentration of heparin was very similar to that reported here for enhanced PGE and collagenase production by ASC. Although mononuclear-cell cultures usually contain more than 90% monocyte/macrophages, there are some T-lymphocytes present. It is known that the T-lym-

phocytes release a soluble product that modulates the production of MCF by the monocyte/macrophages (Krane, 1981; Amento *et al.*, 1982), and that sulphated polyanions such as heparin are T-cell mitogens (Sugawara & Ishizaka, 1982). Therefore it is possible that heparin could either stimulate the production of this lymphocyte factor, act directly on the macrophage, or might affect both cell types.

The bioassay of measuring collagenase and PGE production from synovial fibroblast cultures has several shortcomings for the quantification of MCF. The most important is knowing whether maximal stimulation of collagenase or prostaglandin synthesis has been attained. In the present study the addition of MCF was shown to be within a range that would permit an assessment of relative amounts of monokine.

The interaction of MCP and monocyte/macrophages to produce increased amounts of MCF could have profound effects on inflammatory processes *in vivo*. The monokine is known to stimulate production of PGE, collagenase and other neutral proteinases by a variety of mesenchymal cells including fibroblasts, chondrocytes, tumour cells and corneal stromal cells (for review, see Woolley *et al.*, 1984). Such an interaction could be of importance at sites of cartilage erosion in rheumatoid joints, especially as immunoreactive collagenase (Woolley *et al.*, 1977) and mast cells (Bromley & Woolley, 1984; Bromley *et al.*, 1984) have been reported in these locations. Increased numbers of mast cells have also been associated with areas of infiltration by malignant tissue in human breast cancer (Hartveit, 1981) and mouse skin cancer (Van den Hooff & Tigchelaar-Gutter, 1983), observations that might reflect either a direct stimulation of effector cells or activation of collagenolysis via MCF (Henry *et al.*, 1983).

There is increasing evidence that mast cells are implicated in pathological bone remodelling, and heparin has been implicated (Goldhaber, 1965), although its mechanism of action remains speculative (Crisp *et al.*, 1984). In view of the recent observations that MCF stimulates bone resorption *in vitro* (Gowen *et al.*, 1983*a*,*b*), it is possible that mast-cell heparin could induce bone resorption, at least in part, by its ability to stimulate production of MCF.

The data reported here suggest that mast-cell–macrophage interactions could play a prominent role in the pathogenesis of inflammation and might well contribute to the proteolytic mechanisms involved in connective-tissue degradation.

We thank Mr. Keith Ward for provision of dog mastocytomas, and consultant orthopaedic surgeons (Mr. H. Bertfield, Mr. M. Morris, and Mr. H. Williams) for the supply of rheumatoid synovia. We also thank Mrs. M. Williamson for preparing the typescript. This work was supported by the Oliver Bird Fund of the Nuffield Foundation, and in part by the Medical Research Council and Arthritis and Rheumatism Council.

References

Amento, E. P., Kurnick, J. T., Epstein, A. & Krane, S. M. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5307–5311

Baggiolini, M., Horisberger, U. & Martin, U. (1982) *Int. Arch. Allergy Appl. Immunol.* **67**, 219–226

Barrett, M. D. & Metcalfe, D. D. (1984) *Biochem. Pharmacol.* **33**, 2563–2568

Bleiberg, I., MacGregor, I. & Aronson, M. (1983) *Thromb. Res.* **29**, 53–61

Bromley, M. & Woolley, D. E. (1984) *Arthritis Rheum.* **27**, 857–863

Bromley, M., Fisher, W. D. & Woolley, D. E. (1984) *Ann. Rheum. Dis.* **43**, 76–79

Camussi, G., Tetta, C., Bussolino, F., Masera, C., Emanuelli, G., Ragni, R. & Porcellini, G. (1980) *Panminerva Med.* **22**, 117–124

Cawston, T. E. & Barrett, A. J. (1979) *Anal. Biochem.* **99**, 340–345

Crisp, A. J., Chapman, C. M., Kirkham, S. E., Schiller, A. L. & Crane, S. M. (1984) *Arthritis Rheum.* **27**, 845–851

Dayer, J. M., Passwell, J. H., Schneeberger, E. E. & Krane, S. M. (1980) *J. Immunol.* **124**, 1712–1720

Farndale, R. W., Sayers, C. A. & Barrett, A. J. (1981) *Connect. Tissue Res.* **9**, 247–248

Godfrey, H. P., Ilardi, C., Engber, W. & Graziano, F. M. (1984) *Arthritis Rheum.* **27**, 852–857

Goldhaber, P. (1965) *Science* **147**, 407–409

Gowen, M., Wood, D. D., Ihrie, E. J., McGuire, M. K. B. & Russell, R. G. G. (1983*a*) *Nature (London)* **206**, 378–380

Gowen, M., Meikle, M. C. & Reynolds, J. T. (1983*b*) *Biochim. Biophys. Acta* **762**, 471–474

Hartveit, F. (1981) *J. Pathol.* **134**, 7–11

Henry, N., Eeckhout, Y., Van Lamsweerde, A. L. & Vaes, G. (1983) *FEBS Lett.* **161**, 243–246

Janes, J. & McDonald, J. R. (1948) *Arch. Pathol.* **45**, 622–634

Jaques, L. B. (1979) *Science* **206**, 528–533

Krane, S. M. (1981) *Ann. Rheum. Dis.* **40**, 433–448

Levine, L., Gutierrez-Cernosek, R. M. & Van Vunakis, H. (1971) *J. Biol. Chem.* **246**, 6782–6785

Lindahl, U., Pertoft, H. & Seljelid, R. (1979) *Biochem. J.* **182**, 189–193

Mencia-Huerta, J. M. & Beneveniste, J. (1979) *Eur. J. Immunol.* **9**, 409–415

Menninger, H., Putzier, R., Mohr, W., Wessinghage, D. & Tillmann, K. (1980) *Z. Rheumatol.* **39**, 145–156

Mizel, S. B., Dayer, J. M., Krane, S. M. & Morgenhagen, S. E. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2474–2477

Razin, E., Stevens, R. L., Austen, K. F., Caulfield, J. P., Hein, A., Liu, F. T., Clabby, M., Nabel, G., Cantor, H. & Friedman, S. (1984) *Immunology* **52**, 563–575

Schultz, R. M., Papamatheakis, J. D. & Chirigos, M. A. (1977) *Science* **197**, 674–676

Schorlemmer, H. U., Burger, R., Hylton, W. & Allison, A. C. (1977) *Clin. Immunol. Immunopathol.* **7**, 88–96

Shore, P. A., Burkhalter, A. & Cohn, U. H. (1959) *J. Pharmacol. Exp. Ther.* **127**, 182–187

Sugawara, I. & Ishizaka, S. (1982) *Cell Immunol.* **74**, 162–171

Van den Hooff, A. & Tigchelaar-Gutter, W. (1983) *Med. Proc.* **C86(2)**, 179–190

Woolley, D. E., Crossley, M. J. & Evanson, J. M. (1977) *Arthritis Rheum.* **20**, 1231–1239

Woolley, D. E., Glanville, R. W., Roberts, D. R. & Evanson, J. M. (1978) *Biochem. J.* **169**, 265–276

Woolley, D. E., Brinckerhoff, C. E., Mainardi, C. L., Vater, C. A., Evanson, J. M. & Harris, E. D., Jr. (1979) *Ann. Rheum. Dis.* **38**, 262–270

Woolley, D. E., Taylor, D. J. & Yoffe, J. R. (1984) *Life Chem. Rep.* **2**, 181–236

Yoffe, J. R., Taylor, D. J. & Woolley, D. E. (1984) *Biochem. Biophys. Res. Commun.* **122**, 270–276