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MAST CELL PRODUCTS STIMULATE COLLAGENASE AND PROSTAGLANDIN E PRODUCTION BY CULTURES OF ADHERENT RHEUMATOID SYNOVIAL CELLS

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Mast cells were purified from histologically-confirmed dog mastocytomas and extracted for whole mast cell products (MCP). When added to cultures of human adherent rheumatoid synovial cells MCP induced a 50-400 fold increase in prostaglandin E synthesis and a 10-50 fold stimulation of collagenase production. The mast cell stimulatory factor has not been identified and was not due to histamine, heparin or prostaglandin E. These results indicate a novel way in which mast cells might interact with synovial cells to promote the production of inflammatory mediators and proteolytic enzymes which might contribute to connective tissue degradation.

An increased incidence of mast cells in rheumatoid synovial tissues has been reported (1-4) and recent histochemical studies have demonstrated local accumulations of mast cells at sites of cartilage erosion (5). Their precise role in the pathophysiology of the rheumatoid lesion has yet to be elucidated, but they are known to play important roles in the mediation of local homeostasis and other inflammatory disorders (6).

Adherent rheumatoid synovial cells (ASC) have been shown to produce collagenase and prostaglandins in vitro, especially when cultured in the presence of monocyte-macrophages or conditioned medium containing mononuclear cell factor (7). Similar studies have confirmed that monocytes and macrophages produce soluble factors akin to interleukin 1 that greatly enhance the production of neutral proteinases and prostaglandins from skin fibroblasts (8), chondrocytes (9) and tumor cells (10), demonstrating the importance of cell:cell interactions in the regulation of prostaglandin and proteinase biosynthesis.

We have used in vitro techniques to examine the effects of mast cell products on collagenase and prostaglandin expression by adherent rheumatoid

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synovial cells. We report here that mast cell:synovial cell interactions may contribute to the degradative and inflammatory events which are common features of the rheumatoid lesion.

MATERIALS AND METHODS

Preparation of mast cell products.

Mast cells were prepared from three histologically confirmed dog mastocytomas by mincing and repeated washing with $Ca^{++}Mg^{++}$-free Hank's balanced salt solution (HBSS). Mast cell suspensions were prepared at $5x10^6$cell/ml in Dulbecco's modified Eagles medium (DMEM) and shown to contain >90% mast cells, as judged by metachromatic staining with toluidine blue. Mast cell products (MCP) were prepared by sonication and extraction with 1M NaCl at 4°C overnight (11). Granular debris was removed by centrifugation at 36,000 g for 45 minutes and Millipore filtration. MCP was diluted to the equivalent of 10^6cell/ml. A similar extract was prepared from two dog tumors which did not contain mast cells, and also from human peripheral blood mononuclear cells (10^5 cells/ml).

Histamine and heparin were assayed using the o-phthalaldehyde assay (12) and the dimethyl-methylene blue assay (13), respectively. Prostaglandin E was measured using conventional radioimmunoassay. Neutral proteinase activity was assayed using ^{3}H-labelled human haemoglobin as substrate.

Preparation of mononuclear cell factor.

Human peripheral blood mononuclear cells (10^7 cells/ml) were cultured in DMEM supplemented with 10% fetal calf serum (FCS) for 72h as described previously (7), to provide conditioned medium (MCCM) containing mononuclear cell factor (MCF).

Synovial cell culture.

Fresh rheumatoid synovium obtained from remedial synovectomies was dissociated enzymatically as described previously (7). Adherent cells were cultured in DMEM/10% FCS and antibiotics in 12-well plastic cluster dishes (Costar). Cultures were incubated at 37°C in a water-saturated atmosphere of 5% CO_2 in air. The adherent synovial cells (ASC) of the primary culture contained variable proportions of fibroblasts, macrophages and dendritic cells, but after subculture the passaged cells exhibited a uniformly fibroblastic appearance. Confluent monolayers of ASC were incubated with DMEM/10% FCS with and without substances under investigation. Medium was collected after 24 hours and subsequently assayed for PGE. The cells were washed 3 times with HBSS and test incubations continued for a further 48 hours in serum-free DMEM prior to collagenase assay. Experiments were repeated at least 3 times and results from one representative experiment are shown.

Collagenase activity was determined using C^{14}-glycine-labelled collagen in the fibril assay (14). Latent collagenase was activated by trypsin treatment (10µg/ml) for 20min at room temperature followed by the addition of soybean trypsin inhibitor (50µg/ml). 1 unit of collagenase activity is equivalent to 1µg of collagen degraded/ml/h at 37°C. MCP, non-mast cell extracts and MCCM were added to ASC cultures at a dilution of 1:10. Histamine dihydrochloride (Sigma) was used at 1 to 30µg/ml, heparin (Sigma, bovine mucosal Grade 1) at 5 to 100µg/ml and PGE (Sigma) at 25ng/ml.

RESULTS AND DISCUSSION

Human mast cells are difficult to purify due to their sparse distribution and heterogeneity with respect to size, density, maturity and content (15,16).

TABLE 1. COMPARATIVE ANALYSIS OF MAST CELL PREPARATIONS (10^6 cell/ml).

	MAST CELL PREPARATIONS			TUMOR CELL PREPARATIONS	
	A	B	C	D	E
MORPHOLOGY & TOLUIDINE BLUE	+	+	+	-	-
HISTAMINE (μg/ml)	11	4.5	37	0.9	0.2
HEPARIN (μg/ml)	40	30	240	7	5
NEUTRAL PROTEINASE (μg^3H-Hb/ml/hr)	37	46	89	6	5
PROSTAGLANDIN E (ng/ml)	250	3.6	150	4	0.6

Canine mastocytomas were used as an alternative source as they provide pure preparations of mast cells which are more closely akin to the human equivalent than rat peritoneal mast cells (6,17).

MCP prepared from purified mast cells derived from 3 histologically confirmed mastocytomas (A, B and C) all contained histamine, heparin, neutral proteinase(s) and PGE (Table 1). The variations in quantity and relative proportions of each MCP component is probably explained by the heterogeneity of mast cells. The extracts prepared from dog tumors which were not mastocytomas (D and E) contained very little of these substances. They, and the mononuclear cell extract (F), were included as controls to assess the specific effects of MCP.

Primary cultures of ASC produced large amounts of PGE and latent collagenase. MCP caused a slight increase in PGE production and also increased the amount of active collagenase produced, but had no effect on total collagenase production (data not shown). This is probably explained by the fact that primary ASC are already fully activated by the presence of macrophages (7).

Subcultured ASC (second to fourth passage) with very low basal production of PGE and collagenase were stimulated by all three MCP preparations with a 50 to 400-fold increase in PGE production. In contrast the two non-mast cell tumor extracts had no effect (Fig.1). The MCP preparations also caused a 10 to 50-fold increase in total collagenase production by ASC, whereas non-mast cell

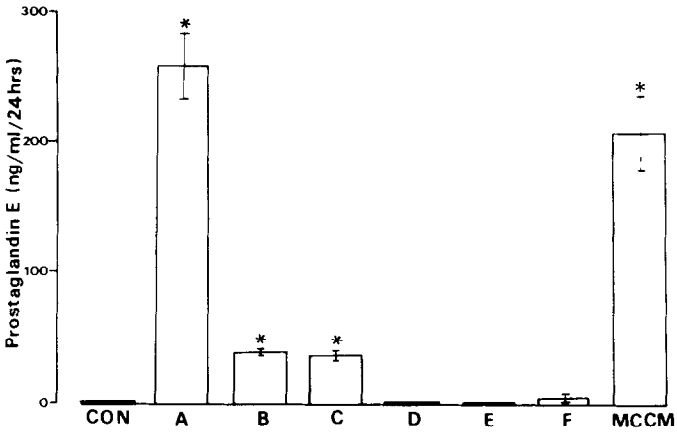

Figure 1. **Effect of mast cell products on prostaglandin E production by passaged adherent synovial cells.**

Confluent monolayers of passaged ASC (p3) were treated with MCP (A,B,C), non-mast cell tumor extracts (D,E), mononuclear cell extract (F) and MCCM, all at 10% as described in Methods. Results are mean values ± S.E.M. for quadruplicate determinations with MCP-derived PGE subtracted. *$p < 0.002$ (Student's t-test).

extracts had negligible effect (Fig.2). The observation that the mononuclear cell extract (F) had no effect on PGE or collagenase production indicates that the MCP stimulating factor is not attributable to the very small numbers of

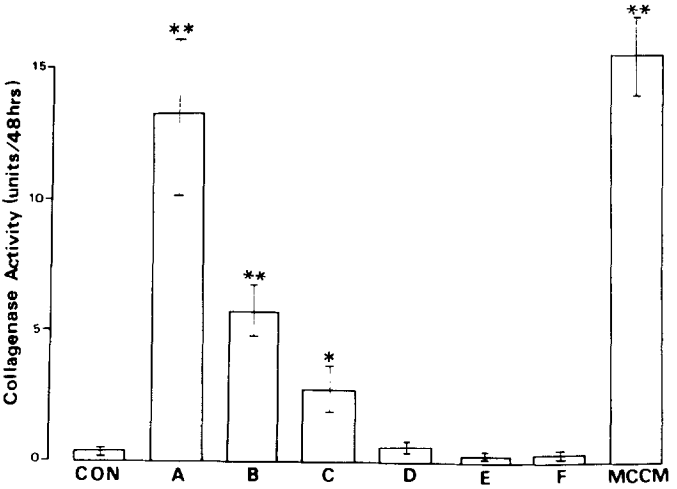

Figure 2. **Effect of mast cell products on collagenase production by passaged adherent synovial cells.**

Confluent monolayers of passaged ASC (p3) were treated with MCP (A,B,C), non-mast cell tumor extracts (D,E), mononuclear cell extract (F) and MCCM, all at 10% as described in Methods. Results are mean values ± S.E.M. for quadruplicate determinations. *$p < 0.05$. **$p < 0.01$ (Student's t-test).

monocyte-macrophages that may be present in the mast cell preparations. MCCM containing mononuclear cell factor was found to stimulate both PGE and collagenase production as expected (Figs. 1 and 2). The degree of stimulation was similar to that observed for the most effective MCP preparation, and when allowance was made for cell numbers the stimulatory potential of mast cells and monocyte-macrophages were of a similar order.

Commercial preparations of histamine (1 to 30μg/ml), heparin (5 to 100μg/ml) and PGE 25ng/ml had no detectable effect on PGE or collagenase production by ASC (data not shown), indicating that these components were not the stimulatory factor(s).

This study demonstrates that dog mast cells contain a factor (or factors) which stimulates subcultured adherent synovial cells to produce increased amounts of PGE and collagenase. The stimulatory factor was found only in extracts of cells characterised as mast cells histologically and biochemically. The identity of the stimulatory factor is uncertain but histamine, heparin and PGE can be eliminated as each failed to produce a significant response at similar or higher concentrations to that of the MCP preparations. Moreover, the extent of stimulation was unrelated to the histamine, heparin or PGE content of each MCP preparation. As the identified constituents of mast cell granules account for less than half the total estimated protein it is highly probable that mast cells contain factors with hitherto undefined activities (19).

The endogenous proteinase activity of MCP was inhibited by titration against fetal calf serum prior to culture. This provided a working compromise that permitted inhibition of the mast cell proteinases but allowed expression of the stimulated ASC collagenase. Although we have shown that mast cells have no extractable collagenase (data not shown) their involvement in collagen degradation has been indicated by several studies. For instance the degradation of rat mesenteric collagen fibres originated exclusively around degranulated mast cells (18), and more recently a regulatory role for mast cells in the collagenolytic activity of tumour invasion was proposed (19).

Phagocytosis of rat mast cell granules by fibroblasts was reported to increase the release of collagenase and lysosomal hydrolase, possibly through a phagocytic stimulus (20). Our observation that MCP, with phagocytosable particles removed during preparation, can stimulate PGE and collagenase production by ASC indicates that soluble mast cell factors are probably the primary stimulus.

The findings that dog mast cells contain a soluble factor (or factors) that stimulates collagenase and PGE production by adherent synovial cells in culture indicates a novel way in which mast cells could participate in connective tissue degradation. Such cellular interactions in vivo might stimulate and perpetuate both the inflammatory response and the proteolytic mechanisms involved in the degradation of cartilage and other matrices.

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