

# Effects of induced mast cell activation on prostaglandin E and metalloproteinase production by rheumatoid synovial tissue in vitro

L C Tetlow, N Harper, T Dunningham, M A Morris, H Bertfield, D E Woolley

## Abstract

**Objective**—To determine whether induced mast cell activation/degranulation in rheumatoid synovial explants modulates the production of prostaglandin E (PGE<sub>2</sub>), and the matrix metalloproteinases (MMPs) collagenase 1, gelatinase A, and stromelysin 1.

**Methods**—Synovial explant cultures were treated either with rabbit IgG anti-human IgE as a mast cell (MC) secretagogue or with non-immune rabbit IgG as controls. After 20 hours conditioned medium was assayed for the release of MC tryptase, PGE<sub>2</sub>, collagenase 1, gelatinase A, and stromelysin 1 using radioimmunoassay, enzyme linked immunosorbent assay, western blot, and zymogram techniques; tissue explants were examined immunohistologically for the relative distributions of MC tryptase, collagenase 1, and stromelysin 1.

**Results**—Over a 20 hour incubation period the MC secretagogue treated explants showed a significant increase in the quantities of released tryptase and PGE<sub>2</sub> compared with controls. By contrast, the three MMPs showed variable values between experiments in response to MC activation; no reproducible trend of either an increased or decreased production of each MMP over control values was evident. Each MMP initially appeared as an inactive precursor form; collagenase 1 and stromelysin 1 were more effectively processed to active forms in the MC activated cultures. Immunolocalisation studies of MC activated explants showed that areas of extracellular tryptase were commonly associated with the local production of both collagenase 1 and stromelysin 1.

**Conclusion**—MC degranulation induced artificially in rheumatoid synovial explant cultures consistently resulted in an increased production of PGE<sub>2</sub>, but had variable effects on the quantification of released collagenase 1, gelatinase A, and stromelysin 1. Such observations support the concept that activated synovial MCs within their native environment stimulate the production of non-MC derived PGE<sub>2</sub> and may contribute to the regulation and processing of specific MMPs; both aspects represent important components of the inflammatory and degradative processes of the rheumatoid lesion.

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Recent studies have drawn attention to the potential importance of mast cells (MCs) in the pathophysiological processes associated with joint destruction in rheumatoid arthritis.<sup>1-5</sup> Mast cells are considered to play a pivotal part in hypersensitivity reactions, angiogenesis, inflammation, and tissue remodelling.<sup>6-8</sup> Their ability to release numerous potent mediators, including histamine, heparin, tryptase, chymase, leukotrienes, cytokines, and growth factors, indicates that activated MCs are likely to bring about changes in the behaviour of all neighbouring cells, as well as producing oedema and disruption of the connective tissue architecture.<sup>1,7,9</sup> Increased numbers of MCs are found in the synovial tissue and fluids of RA joints; and MC activation or degranulation has been demonstrated at sites of cartilage erosion in a significant proportion of rheumatoid specimens.<sup>9,10</sup> Moreover, MCs were identified in all specimens of rheumatoid synovial tissue, but their distribution and local concentrations varied, both within and between specimens.<sup>3,9</sup> Observations of MC degranulation in some specimens have been colocalised with the production of the proinflammatory cytokines interleukin 1 and tumour necrosis factor  $\alpha$ , as well as the matrix degrading enzymes collagenase 1 and stromelysin 1.<sup>1</sup> Such findings suggested that MC activation could well contribute to the degradative processes associated with cartilage loss.<sup>1,2,5,10</sup>

Rheumatoid synovial tissue in explant culture produces significant amounts of prostaglandin E and a variety of proteinases, including the cartilage degrading matrix metalloproteinases (MMPs) collagenase 1, gelatinase A, and stromelysin 1.<sup>11,12</sup> Our recent studies have shown that the production of collagenase 3, an enzyme that preferentially attacks cartilage collagen type II, is absent or at best negligible compared with collagenase 1 in rheumatoid synovial tissue.<sup>13</sup> Collagenase 1 is the rate limiting enzyme for the degradation of fibrillar collagens; gelatinase A degrades collagens IV, V, VII, X, elastin, and fibronectin; whereas stromelysin 1 attacks various components of the cartilage matrix such as aggrecan and some cartilage collagens.<sup>11,12,14,15</sup> The production of collagenase 1 and stromelysin 1 by synoviocytes or chondrocytes is stimulated by the proinflammatory cytokines interleukin 1 (IL1) and tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), these purportedly being expressed mainly by activated macrophages,<sup>16,17</sup> although MCs are also a source of TNF $\alpha$ .<sup>18,19</sup> Earlier in vitro studies

University  
Department of  
Medicine, Manchester  
Royal Infirmary,  
Manchester  
L C Tetlow  
N Harper  
D E Woolley

Tameside General  
Hospital, Manchester  
T Dunningham

Devonshire Royal  
Hospital, Buxton,  
Derbyshire  
M A Morris

Wythenshawe Hospital,  
Manchester  
H Bertfield

Correspondence to:  
Dr D E Woolley, University  
Department of Medicine,  
Manchester Royal Infirmary,  
Oxford Road, Manchester  
M13 9WL.

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have shown that the addition of soluble products from MCs stimulated cytokine production by monocyte-macrophages<sup>20</sup> and also stimulated collagenase and prostaglandin E production by synovial fibroblasts.<sup>21</sup> Moreover, MC products not only stimulate cells to produce the MMPs, but the MC specific enzymes tryptase and chymase also have the ability to activate the precursor forms of these MMPs.<sup>22-24</sup>

MC activation or degranulation may be induced in vivo by several mechanisms; although the antigen IgE mediated process is well understood in allergy, soluble products from a variety of cell types including T cells, monocyte-macrophage, granulocytes, and platelets are also effective, in addition to some drugs and neuropeptides.<sup>6,7</sup> In vitro studies of MC activation have commonly used secretagogues such as 48/80, the calcium ionophore A23187, or antibodies to IgE.<sup>4,25</sup> The latter, because of its better specificity for MCs, has been used in this study to induce MC degranulation in explant cultures of rheumatoid synovium to examine the effects of MC activation on prostaglandin E and metalloproteinase production.

## Methods

### TISSUE CULTURE

Rheumatoid synovium was obtained from patients with classic rheumatoid arthritis undergoing remedial synovectomy or total knee replacement operations. The rheumatoid synovial specimens used in this study were derived from knee synovectomies (specimens 674, 700, 798) or knee arthroplasties (specimens 689, 691, 763, 770, 773). All specimens, despite recognised cellular heterogeneity<sup>3,9</sup> showed variable content of inflammatory cells, as determined histologically. End stage, fibrotic or "burnt-out" specimens were excluded from this study. Synovial tissue was separated from fat, bone, cartilage, and fibrous tissues and was cut into small pieces of approximately 3 mm<sup>3</sup>. After washing in Hanks's balanced salt solution (Gibco, Paisley, Scotland) 18–20 randomised explants (about 400 mg) were placed in a pre-weighed sterile culture dish. After re-weighing to determine the tissue weight per dish, 2 ml of Dulbecco's modified Eagle's medium (DMEM, Gibco, Paisley, Scotland) containing either rabbit IgG antibody to human IgE (DAKO, Glostrup, Denmark) or control rabbit IgG (CRIgG) each at a concentration of 150 µg/ml was added to each culture dish in duplicate or triplicate. The dishes were incubated at 37°C in a 5% CO<sub>2</sub>/95% air incubator with humidified atmosphere. Culture medium was removed at time intervals six hours, 20 hours, and 44 hours and replaced with fresh medium supplemented with the immunoglobulins as described. Each sample of conditioned culture medium was aliquoted and stored at -20°C until being assayed for MC tryptase, PGE<sub>2</sub>, collagenase 1, gelatinase A, and stromelysin 1.

### TRYPTASE ASSAY

Samples were assayed for MC tryptase using tryptase RIACT immunoradiometric assay kits

from Pharmacia (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden). Final results were calculated as ng tryptase/ml/100 mg tissue.

### PROSTAGLANDIN E<sub>2</sub> ASSAY

Samples were assayed for PGE<sub>2</sub> using a monoclonal enzyme immunoassay kit from Cayman Chemicals (supplied by SPI BIO, France). Final results were calculated as ng PGE<sub>2</sub>/ml/100 mg tissue.

### COLLAGENASE 1 ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Collagenase 1 was measured using an ELISA assay based on methodology described previously.<sup>26</sup> The assay used a monospecific rabbit polyclonal anti-human collagenase 1 as the capture antibody and a monospecific biotinylated sheep anti-collagenase 1 antibody for antigen detection. Horseradish peroxidase (HRP) linked to streptavidin was developed using 3,3',5,5'-tetramethyl benzidine (TMB) substrate for quantification in a Dynatech plate reader. The sensitivity range for the collagenase 1 ELISA was 0.8–50 ng/ml for the precursor form; but when fully activated (1.5 mM APMA, 16 hours, 37°C)<sup>24</sup> an equivalent amount of active enzyme produced an absorbance value about 50% that of the precursor. Final results were calculated as ng collagenase 1/ml/100 mg tissue.

### STROMELYSIN 1 ELISA

Stromelysin 1 was assayed using a double antibody ELISA system developed within the laboratory.<sup>26</sup> The 96 well plates were coated with a monospecific rabbit polyclonal anti-human stromelysin 1 antibody as the capture antibody, with a monospecific biotinylated sheep polyclonal anti-stromelysin 1 antibody for antigen detection. Streptavidin linked HRP was developed with TMB substrate. The sensitivity range for the stromelysin 1 ELISA was 0.4–25 ng/ml for the precursor form; but when fully activated (1.5 mM APMA, 22 hours, 37°C)<sup>24</sup> an equivalent amount of active enzyme produced an absorbance value about 10% that of the precursor. Final results were calculated as ng stromelysin 1/ml/100 mg tissue.

All antibodies used in the ELISA assays were monospecific and showed no cross reactivities when tested by western blotting against collagenase 1, stromelysin 1, gelatinase A and B.<sup>23,26</sup> Antibodies were generously provided by Drs D J Taylor and M Lees, Department of Medicine, Manchester Royal Infirmary.

### GELATINASE A ELISA

Gelatinase A was measured using ELISA kits purchased from The Binding Site, Birmingham, UK.

### WESTERN BLOTS

Western blotting was carried out as previously described.<sup>23,24</sup> Samples were separated by SDS-PAGE under non-reducing conditions and were electrotransferred to Zetaprobe GT nylon membrane (BioRad, Richmond, CA). The primary antibodies, sheep anti-(human) collagenase 1, and sheep anti-(human) stromelysin 1

were used at dilutions 1:500 and 1:1000, respectively. The secondary anti-sheep antibodies were biotin conjugated and were used at dilutions of 1:1000, followed by linkage to streptavidin conjugated HRP (all from DAKO, Glostrup, Denmark). Colour development was achieved using the chromogenic substrate 3,3'-diaminobenzidine tetrahydrochloride (DAB) for peroxidase.

#### ZYMOGRAPHY

Enzyme samples were separated by SDS-PAGE on 7.5% (mass/vol) polyacrylamide gels, containing gelatin at 1 mg/ml, under non-reducing conditions. After washing for one hour at room temperature in 50 mM TRIS/HCl, pH 7.5, containing 5 mM CaCl<sub>2</sub>, 1 μM ZnCl<sub>2</sub>, 0.02% (mass/vol) NaN<sub>3</sub> and 2.5% (v/v) Triton X-100, the gels were incubated in the same buffer containing 1% (v/v) Triton X-100 for 16 hours at 37°C and stained with Coomassie brilliant blue. Gelatinolytic activity was detected by negative staining.

#### METALLOPROTEINASE STANDARDS

Standards for collagenase 1, gelatinase A, and stromelysin 1 were purified as described previously.<sup>23</sup>

#### IMMUNOHISTOCHEMISTRY

Explant cultures in parallel to those set up as described were sampled at six hours, 20 hours, and 44 hours. All tissues were fixed in Carnoy's fixative for one hour, dehydrated in absolute ethanol, and conventionally embedded in paraffin wax. Five μm sections were cut, dewaxed, rehydrated, and examined for the presence of MC tryptase, collagenase 1 and stromelysin 1 using immunolocalisation techniques as previously described.<sup>1</sup>

**Table 1** Tryptase release by explants of rheumatoid synovium after 20 hours with and without MC activation by anti-IgE. Values are given as ng/ml/100 mg tissue

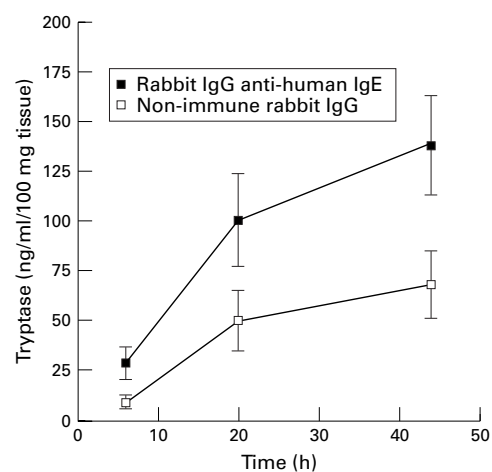
Specimen no	Control values	Secretagogue treated	Relative change (x)
674	29.13	56.7	2.0
689	152.03	202.5	1.3
691	50.6	55.0	1.1
700	79.27	145.57	1.8
763	62.4	147.63	2.4
770	8.63	46.6	5.4
773	8.88	47.9	5.4
798	32.3	42.08	1.3

The secretagogue treated values were significantly higher (paired *t* test, *t*(7)=4.2; *p*=0.004).

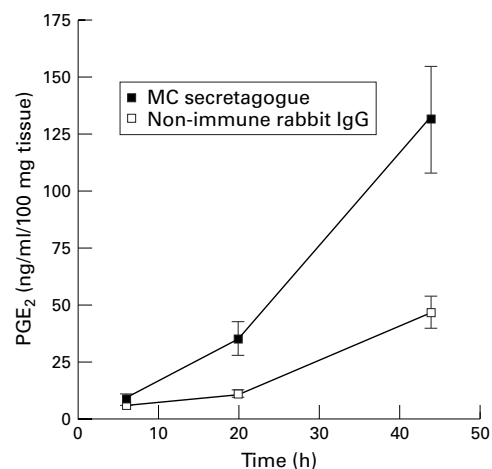
**Table 2** PGE<sub>2</sub> production by explants of rheumatoid synovium after 20 hours in culture with and without MC activation by anti-IgE. Values are given as ng/ml/100 mg tissue

Specimen no	Control values	Secretagogue treated	Relative change(x)
674	6.5	10.8	1.7
689	8.5	48.5	5.7
691	13.8	32.3	2.3
700	10.3	23	2.2
763	15.9	22.3	1.4
770	26.1	38.9	1.5

The secretagogue treated values were significantly higher (Wilcoxon matched pairs rank test, *z*=2.2; *p* = 0.028).



**Figure 1** Cumulative data for tryptase release from rheumatoid synovial explant cultures treated with MC secretagogue or non-immune rabbit IgG over 44 hours. Data points represent mean (SEM) values from five different specimens.



**Figure 2** Cumulative data for PGE<sub>2</sub> release from rheumatoid synovial explant cultures treated with MC secretagogue or non-immune rabbit IgG. Data points represent mean (SEM) values from four different specimens.

#### IMMUNOLocalISATION OF MC TRYPTASE

Tissue sections were dewaxed and pretreated for 30 minutes with goat serum at 10% v/v in TRIS buffered saline (TBS) pH 7.6. Mouse monoclonal antibody to human MC tryptase (Chemicon International, London) was diluted 1:300 in TBS and applied to tissue sections for two hours at 20°C. After three 10 minute washes in TBS, alkaline phosphatase (AP) conjugated goat antimouse IgG (DAKO Ltd, UK) diluted 1:50 was applied for one hour at 20°C. After further washing the AP was developed using New Fuchsin substrate. Tissue sections were lightly counterstained using Harris's haematoxylin. For controls, TBS or normal mouse IgG was substituted for the primary antibody on tissue sections and each consistently gave negative findings.

#### IMMUNOLocalISATION OF COLLAGENASE 1 OR STROMELYSIN 1

Tissue sections were pretreated with normal donkey serum diluted 1:10 in TBS for 30 minutes. Affinity purified sheep polyclonal

Table 3 (A) Production of collagenase 1 at 20 hours by explants of rheumatoid synovium with and without MC activation by anti-IgE. Values given are in ng/ml/100 mg tissue

Specimen no	Control values	Secretagogue treated	Relative change (x)
674	13.2	10.56	0.8
689	12.4	28.1	2.3
691	19.0	64.2	3.4
700	12.8	17.1	1.3
763	69.3	85.2	1.2
770	42.5	14.5	0.3
773	188	143	0.8
798	34	53.5	1.6

There was no significant difference between the control and the secretagogue treated values (paired *t* test,  $t(7)=0.3$ ,  $p=0.77$ ).

Table 3 (B) Production of gelatinase A at 20 hours by explants of rheumatoid synovium with and without MC activation by anti-IgE. Values are given as ng/ml/100 mg tissue

Specimen no	Control values	Secretagogue treated	Relative change (x)
674	20.7	21	1.0
689	56.5	20.3	0.5
691	16.6	23.3	1.4
700	58.8	44.2	0.8
763	23.3	26.8	1.2
770	16.6	18	1.1
773	6.1	10.6	1.7
798	10.8	23.9	2.0

There was no significant difference between the control and secretagogue treated values (Wilcoxon paired rank sum test,  $z=0.4$ ;  $p=0.67$ ).

Table 3 (C) Production of stromelysin-1 at 20 hours by explants of rheumatoid synovium with and without MC activation by anti-IgE. Values are given as ng/ml/100 mg tissue

Specimen no	Control values	Secretagogue treated	Relative change (x)
674	763	1055	1.4
689	1420	1545	1.1
691	442	561	1.3
700	1478	2383	1.6
763	1002	781	0.8
770	499	185	0.4
773	188	143	0.8
798	340	535	1.6

There was no significant differences between the control and the secretagogue treated values, (Wilcoxon paired rank sum test,  $z=0.7$ ;  $p=0.48$ ).

antibodies to either collagenase 1 or stromelysin 1, each at 10 µg/ml, were applied to tissue sections for two hours at 20°C. After three 10 minute washes in TBS, biotin conjugated donkey anti-sheep IgG (DAKO) diluted 1:200 was applied for one hour at 20°C. After further washing the avidin-biotin complex with AP conjugate was applied to the sections for one hour at 20°C and the AP was subsequently developed using New Fuchsin substrate. Sections were lightly counterstained in Harris's haematoxylin. For controls, TBS or normal sheep IgG at concentrations similar to those of the primary antibody were applied to tissue sections and consistently gave negative results.

#### STATISTICAL ANALYSIS

Data for tryptase and collagenase 1 were analysed using a paired Student *t* test. Data for PGE<sub>2</sub>, gelatinase A, and stromelysin 1 did not follow a normal distribution hence a non-parametric test (Wilcoxon rank sum test) was used for analysis.

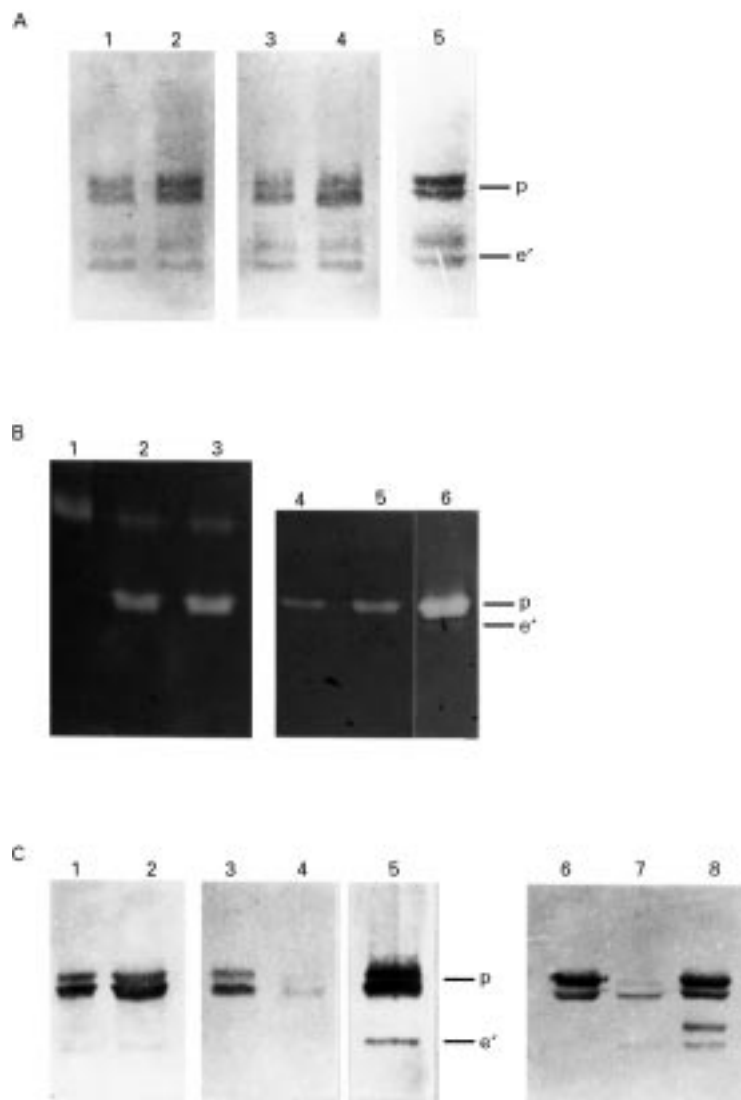
## Results

Measurement of tryptase, an enzyme unique to MCs, was used to assess the extent of MC degranulation in the synovial explant cultures. Rabbit IgG antibody to human IgE was used as the secretagogue; an optimal concentration of 150 µg IgG/ml was determined by measuring the level of tryptase release after six hours in culture. All synovial explant cultures treated with this MC secretagogue showed a consistent increase in tryptase levels over control cultures receiving the same concentration of non-immune rabbit IgG. Figure 1 shows the mean values from five different experiments for the cumulative increase in tryptase release over 44 hours in culture; and table 1 shows the MC tryptase release for eight different experiments after 20 hours of culture with and without secretagogue. It is probable that a proportion of the tryptase released in all cultures reflects some MC degranulation brought about by the physical trauma of explant preparation. However, the data indicate that secretagogue treated cultures consistently released significantly higher tryptase than controls ( $p=0.004$ ), observations confirming an effective induction of MC activation.

Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is a prostanoid not produced by MCs, but mainly by suitably activated synoviocytes and macrophages. Figure 2 shows the cumulative values for PGE<sub>2</sub> release over 44 hours in the control and secretagogue treated cultures, the latter showing an approximate threefold increase in PGE<sub>2</sub> production after 20 hours of culture. Table 2 shows a significant increase in PGE<sub>2</sub> production for each of the secretagogue treated cultures in six different experiments.

We have no evidence from immunolocalisation studies that human MCs produce the MMPs collagenase 1, gelatinase A or stromelysin 1. ELISA measurements of these three enzymes from 20 hour conditioned culture media showed different values for the MC activated cultures compared with controls. Although the replicates used to calculate the mean enzyme values were close and reliable, the secretagogue treated explants either produced an increase or a decrease in MMP production compared with controls (tables 3A, B, and C). Statistical analysis showed no significant difference between overall values for the control and secretagogue treated cultures for each of the three MMPs. However, there may be technical reasons for these variable data.

It is probable that the measurements of these enzymes and PGE<sub>2</sub> in the culture medium do not necessarily reflect total production by the explants, especially as the accessibility of each enzyme and PGE<sub>2</sub> into the culture medium is likely to depend on the relative interactions and binding properties to the explanted tissue matrix, cells, and receptors. In this context, because the MC proteinases tryptase and chymase are known to activate MMP precursors, such activated forms of collagenase 1 and stromelysin 1 may well bind to matrix substrates of the explants and thereby become depleted from the culture medium. Moreover, quantification experiments to evaluate the



**Figure 3** Western blot and zymogram analyses of collagenase 1, gelatinase A, and stromelysin 1 released from rheumatoid synovial explant cultures treated with and without MC secretagogue. (A) Collagenase 1. Western blot analysis of the precursor (p) and active (e') forms of collagenase 1 are shown by the enzyme standard (lane 5). Collagenase 1 released from control cultures is shown after 20 hours (lane 1) and 44 hours (lane 3) for comparison with similar secretagogue treated cultures at 20 hours (lane 2) and 44 hours (lane 4). Note the modest increase in the amounts of precursor enzyme (52 kDa) from the secretagogue treated cultures (lanes 2 and 4) compared with controls, and the presence of active enzyme (43 and 41 kDa) in both the 20 and 44 hour samples. (B) Gelatinase A. Zymography of the precursor (p) and active (e') forms of gelatinase A are shown by the enzyme standard (lane 6); and progelatinase B standard shown on lane 1. Gelatinase A in precursor form released from control cultures after 20 hours is shown in lanes 2 and 4, for comparison with similar secretagogue treated cultures shown in lanes 3 and 5. The precursor form of gelatinase B is also seen in lanes 2 and 3, but no evidence for activation or processing was observed for the gelatinase enzymes. (C) Stromelysin 1. Western blot analysis of the precursor (p) and active (e') forms of stromelysin 1 are shown by the enzyme standard (lane 5). Stromelysin 1 precursor released from control cultures is shown after 20 hours (lane 1) and 44 hours (lane 3) for comparison with similar secretagogue treated cultures at 20 hours (lane 2) and 44 hours (lane 4). Note depletion of the stromelysin 1 precursor in lane 4. The addition of 5  $\mu$ g purified stromelysin 1 precursor (57 kDa; lane 6) was added to 50  $\mu$ l conditioned 44 hour culture medium and secretagogue shown in lane 7. After incubation at 37°C for three hours the sample showed that some of the exogenous precursor had been converted to an active form of stromelysin 1 (45 kDa; lane 8), thereby demonstrating its proteolytic processing capacity for stromelysin 1.

ELISA methodology showed that the precursor forms of the MMPs produced much higher absorbance values than an equivalent amount of active enzyme (see Methods). These findings therefore make it difficult to quantitate the amount or extent of active enzymes produced in explant cultures; but evidence is presented to show that precursor activation occurs for colla-

genase 1 and stromelysin 1 within this culture system.

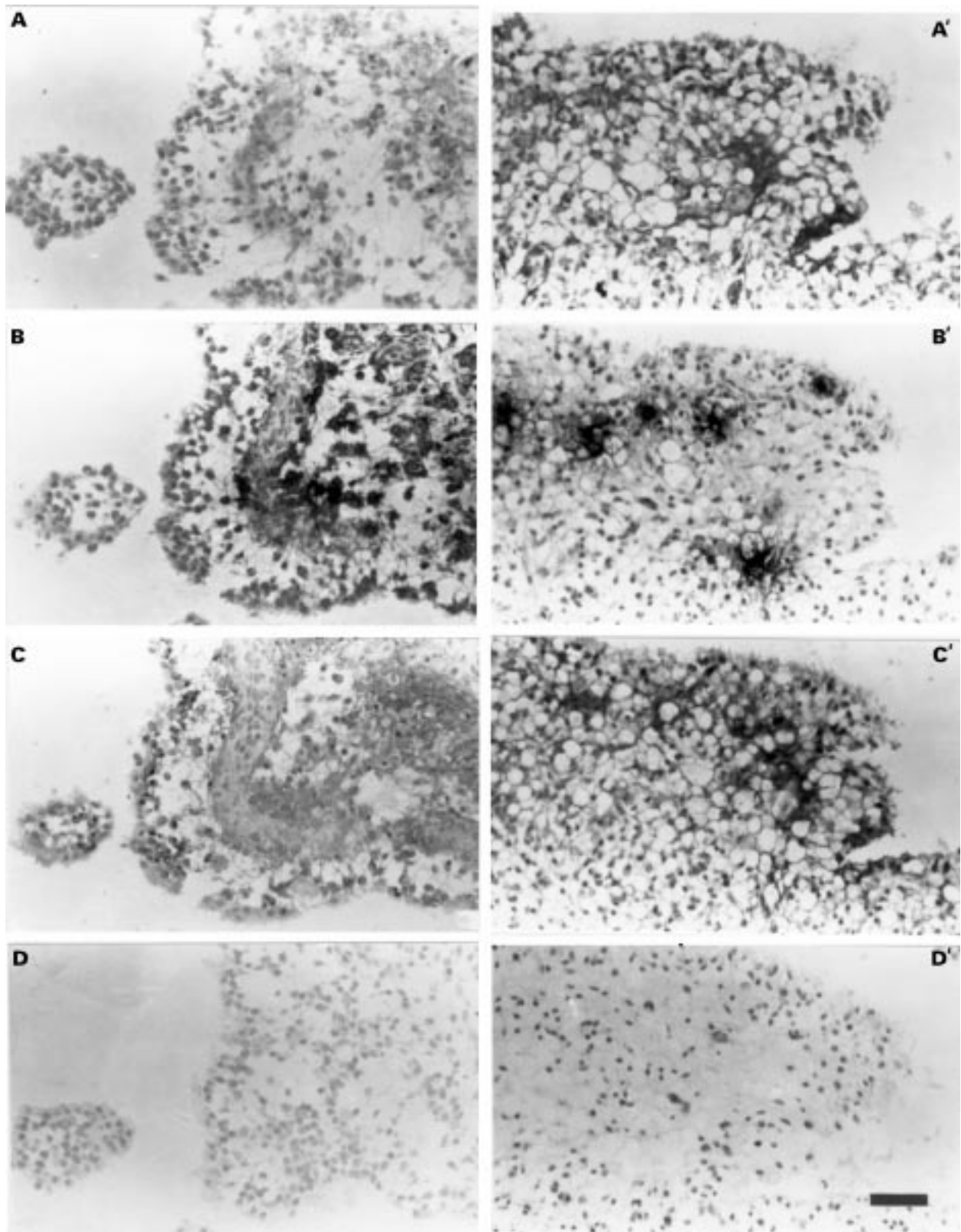
Inactive precursors of collagenase 1, gelatinase A, and stromelysin 1 represent the predominant forms of each enzyme in conditioned medium after 20 hours of culture (fig 3A, B, C). Whereas we found no evidence of conversion of the gelatinase A precursor to the active enzyme in zymogram analysis (fig 3B) of six different experiments, active forms of collagenase 1 (fig 3A) and stromelysin 1 (fig 3C) were observed in most experiments after 20 hours of culture. Moreover, the ability to activate or process the precursors of collagenase 1 and stromelysin 1 increased with time of culture, and especially in the secretagogue treated cultures. For example, the apparent diminution of stromelysin 1 in secretagogue treated cultures relative to controls at later stages of culture is shown to be partly due to the ability of that conditioned medium to process and activate exogenously added stromelysin 1 precursor (fig 3C), probably via the increased availability of the MC serine proteinases. Indeed, conditioned media from control cultures consistently showed negligible or much reduced processing of the exogenous prostromelysin relative to MC activated cultures (data not shown). Such active forms of the MMPs may well bind to tissue substrates of the explants, thereby avoiding quantitative assessment in the culture medium.

Immunolocalisation studies of MC activation, as judged by extracellular tryptase, together with the distribution of collagenase 1 and stromelysin 1, were carried out on secretagogue treated explants fixed after 20 and 44 hours in culture. Consecutive tissue sections were stained for each of the enzymes. At 20 hours extensive extracellular MC tryptase was observed with relatively little staining for collagenase 1, but a proportion of cells and extracellular matrix stained for stromelysin 1 (fig 4). At 44 hours tissue locations showing extracellular MC tryptase were commonly associated with regions immunostained for both collagenase 1 and stromelysin 1 (fig 4). By contrast, tissue areas devoid of MCs usually showed negligible or sparse immunoreactivity for the two MMPs.

## Discussion

This rheumatoid synovial explant study has attempted to obtain information on the functional importance and consequence of mast cell activation *in situ*, specifically with regard to changes in PGE<sub>2</sub> and MMP production and processing. Several studies have isolated synovial mast cells for the characterisation of various secretagogue actions,<sup>27-30</sup> but only recently have synovial explants been used to examine synovial MC function.<sup>4</sup> Data on the use of compound 48/80, calcium ionophore A23187, morphine sulphate, and IgG antibodies to human IgE have been reported,<sup>4 25 30</sup> but the latter was chosen as secretagogue for this study because of its specificity for MCs and lack of action on most other cell types.

Tryptase is the main serine proteinase stored in MC granules; these are released into the



**Figure 4** Immunolocalisation of MC tryptase, collagenase 1, and stromelysin 1 on consecutive tissue sections of rheumatoid synovial explants treated with MC secretagogue after 20 hours (A–D) or 44 hours (A'–D'). Positive immunostaining visualised by the darkly stained cells or matrix compared with control haematoxylin stained tissue sections shown in (D) and (D'). (A) and (A'). Immunolocalisation of collagenase 1. Note very little immunostaining at 20 hours (A) but both intracellular and extracellular staining at 44 hours (A'). (B) and (B') Immunolocalisation of MC tryptase. Both tissue sections at 20 hours (B) and 44 hours (B') show evidence of extracellular tryptase indicative of MC degranulation. Note the association or uptake of tryptase by the lining synovial cells in (B). (C) and (C') Immunolocalisation of stromelysin 1. Note that positive staining is mainly intracellular with weaker staining of matrix at 20 hours (C), whereas at 44 hours widespread extracellular immunostaining is evident (C'). (D) and (D') Haematoxylin stained control sections for (A)–(C), and (A')–(C'), respectively. Bar = 50  $\mu$ m for all micrographs.

extracellular microenvironment after suitable stimulation for degranulation.<sup>7,8</sup> The tryptase enzyme is a tetramer that rapidly loses activity upon conversion to its monomeric form via its dissociation from heparin.<sup>31</sup> MC tryptase has provided not only a useful marker for the immunohistological demonstration of MCs and their degranulation in tissues,<sup>1,9</sup> but its measurement has also been used to monitor MC activation/degranulation both *in vivo* and *in vitro*.<sup>8</sup> Measurement of histamine release has traditionally been the parameter for assessing MC activation in most studies; but as we encountered inconsistencies in measuring histamine released from explants, and because the quantity of histamine receptors and availability of amine oxidase in synovial explants was impossible to determine, measurement of tryptase release was adopted for this study.

While the preparation of tissue explants will inevitably damage some MCs and result in granule release, our tryptase immunohistological studies on control explants showed that MC degranulation, as judged by extracellular tryptase, was mainly confined to cut edges of the tissue explants. By contrast, those exposed to secretagogue showed a generalised MC degranulation throughout the explants, a finding supported by the significant increase of tryptase values in the culture medium. However, as only a proportion of the released tryptase is likely to gain access to the culture medium, much of the enzyme being retained within the explant either by binding to substrates or by phagocytic uptake of MC granules by neighbouring cells,<sup>9,32,33</sup> tryptase concentrations within the culture medium provide only a relative assessment of MC degranulation. The variation in tryptase release observed between different synovial specimens may reflect different MC numbers between specimens, or may be explained by the recent report that rheumatoid synovial MCs are more likely to be in an activated state during clinically active disease.<sup>29</sup>

As the demonstration of extracellular tryptase implies that other MC granule components such as histamine, heparin, chymase, and some cytokines such as TNF $\alpha$  are released concomitantly, it is to be expected that the increased availability of such factors in secretagogue treated cultures would modify other cellular processes, such as prostanoid synthesis and MMP expression.<sup>1,5</sup> This concept was supported by the increased PGE<sub>2</sub> release from explants exposed to MC secretagogue, a finding supporting the earlier report of increased PGE<sub>2</sub> production by cultured synoviocytes exposed to soluble MC products.<sup>21</sup>

Several studies have noted the association of MCs with areas of connective tissue degradation or matrix remodelling.<sup>1,9,34</sup> The codistribution of extracellular MC tryptase with proinflammatory cytokines, MMPs, and oedema in the rheumatoid lesion, as demonstrated by dual immunolocalisation studies, indicated that MCs were associated with the degradative processes within the rheumatoid joint.<sup>1,9</sup> Similar findings of extracellular MC tryptase with associated distributions of collagenase 1 and stromelysin 1 are presented in this study,

thereby adding weight to the concept that MC activation is associated with MMP production by neighbouring cells.<sup>1,2,9</sup> This response is probably explained by the recent realisation that MCs contain or express a variety of cytokines, including TNF $\alpha$  and IL1,<sup>6,8</sup> factors known to stimulate MMP expression by synoviocytes.<sup>16</sup> Indeed, MC products and heparin are known to stimulate proinflammatory cytokine production by monocyte-macrophages,<sup>20</sup> a cell type which together with the synoviocyte is a major cellular component of rheumatoid synovial tissue.<sup>3</sup> Both collagenase 1 and stromelysin 1 are secreted as inactive precursors that require activation.<sup>11,12</sup> The MC proteinases tryptase and chymase have both been demonstrated in the rheumatoid lesion<sup>9</sup> and have recently been shown to activate these proMMPs. Whereas tryptase activates prostromelysin 1, which subsequently activates procollagenase 1,<sup>22</sup> chymase activates the precursors of both collagenase 1 and stromelysin 1.<sup>23,24</sup> By contrast, the precursor form of gelatinase A is not activated by the MC proteinases,<sup>23</sup> a finding supported by the lack of evidence for active gelatinase A in this study.

Although the quantitative data for MMP production in this synovial explant study have given variable results, probably for the reasons outlined in the Results section, it would appear from other related studies that MC activation *in situ* probably contributes to localised matrix degradation,<sup>1,9,34</sup> including the proteolytic potential of the MC<sub>TC</sub> phenotype for other matrix substrates.<sup>35</sup> Experimental evidence clearly points to the rapid release of histamine and its consequential tissue oedema after MC degranulation. However, solubilisation and release of various components from exocytosed granules provides a temporally regulated supply of signals within the extracellular matrix. Furthermore, the synthesis and release of specific cytokines and arachidonate products may follow at specific stages post activation, thereby producing a complex sequence of signals within the localised domain of the activated MC.

This *in vitro* synovial explant study has allowed us to examine some of the consequences of MC activation within its native environment, specifically the effect of MC mediators on the production of PGE<sub>2</sub> and three MMPs, all four of which are not produced by MCs, but mainly by synoviocytes and macrophages. We recognise that control cultures have also shown some degree of MC degranulation because of the physical trauma of explant preparation and possibly the extent of clinical inflammation,<sup>29</sup> and it would be advantageous in future studies to minimise MC activation in the controls. As yet an effective human synovial MC stabilising compound has not been unequivocally identified despite detailed studies of various non-steroidal anti-inflammatory drugs, cyclosporin, and other immunosuppressive compounds on mediator release from isolated human synovial MCs.<sup>36</sup> However, it would seem that this explant methodology would appear suitable for the screening of potential candidates. Once an effective MC stabilising



compound is available its use alongside the anti-IgE secretagogue used in this study should provide more definitive data on the functional significance of MC activation in the rheumatoid lesion.

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