for 36 h. An increase in DNA polymerase $\alpha$ activity observed at 24 h was preceded by an increase in polymerase mRNA at 16 h, indicating that regulation of DNA polymerase $\alpha$ was indeed at the mRNA level. The second peak of mRNA at 32 h may reflect the start of a second round of DNA synthesis by the synchronized cells.

We thank Dr Neil Brown who generously gave the calf thymus cDNA fragment of DNA polymerase $\alpha$. M.R.C. is grateful to the Cancer Research Campaign for provision of a Research Studentship.

Mast cell degranulation results in the release and elaboration of many potent mediators of inflammation. These include granule-derived histamine, neutral proteases and factors chemotactic for eosinophils and neutrophils, and membrane-derived metabolites of arachidonic acid generated by the lipoxgenase and cyclo-oxygenase pathways. Although demonstrable in in vitro systems, most of these mast cell products have been of only limited value as clinical markers of mast cell activation because of their rapid rates of metabolism, or because they are produced by several other types of cell. Recent studies with mast cell neutral proteases suggest that their measurement in body fluids may provide a reliable means of evaluating the extent to which mast cells can participate in disease processes.

The major proteolytic enzyme of the human mast cell has been designated tryptase on account of its tryptic properties [1], and it has been localized to the mast cell in a variety of anatomical sites [2]. Tryptase is present in basophils, but in an amount which is generally less than 1% of that in mast cells [3]. Although the enzymic activity is rapidly lost after release into physiological solutions, the inactive subunits of this tetrameric molecule appear to be relatively stable [4].

We have purified tryptase from homogenized human lung tissue by high salt extraction (2 M-NaCl), 45% (w/v) ammonium sulphate precipitation, octyl-Sepharose column chromatography and heparin-agarose affinity chromatography. The protein isolated has neutral protease activity in an assay using the synthetic substrate $p$-tosyl-L-arginine methyl ester, and a molecular mass of 140 kDa was established by Sephacryl S-200 gel filtration. It consists of four subunits each with a molecular mass of 32 kDa as determined by SDS/polyacrylamide-gel electrophoresis. Our tryptic enzyme thus shares the same physicochemical properties as mast cell tryptase previously described [4, 5]. A yield of approximately 10 mg was obtained from 1 kg of lung.

We have raised rabbit antisera against tryptase and have produced four specific mouse monoclonal antibodies (AM, AA3, AA5). The polyclonal antibody has been confirmed by indirect enzyme linked immunosorbent assay (ELISA), and immunoenzymic overlay techniques. Immunocytochemical procedures with fluorescein or horseradish peroxidase-labelled secondary antibodies indicate that these antibodies bind specifically to the granules of mast cells in cytospin preparations of enzymically dispersed lung, tonsil, colon and skin. Under the same conditions, there is no binding to peripheral blood basophils purified on a Percoll gradient. The monoclonal antibodies are effective also at identifying mast cells in paraffin-embedded tissue.

Human mast cell tryptase: a biochemical marker for mast cell degranulation

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Mast cell degranulation results in the release and elaboration of many potent mediators of inflammation. These include granule-derived histamine, neutral proteases and factors chemotactic for eosinophils and neutrophils, and membrane-derived metabolites of arachidonic acid generated by the lipoxgenase and cyclo-oxygenase pathways. Although demonstrable in in vitro systems, most of these mast cell products have been of only limited value as clinical markers of mast cell activation because of their rapid rates of metabolism, or because they are produced by several other types of cell. Recent studies with mast cell neutral proteases suggest that their measurement in body fluids may provide a reliable means of evaluating the extent to which mast cells can participate in disease processes.

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We have raised rabbit antisera against tryptase and have produced four specific mouse monoclonal antibodies (AM, AA3, AA5 and AA7). The polyclonal antibody has been investigated using quantitative immunoelectrophoretic procedures, and the specificity of the monoclonal antibodies has been confirmed by indirect enzyme linked immunosorbent assay (ELISA), and immunoenzymic overlay techniques. Immunocytochemical procedures with fluorescein or horseradish peroxidase-labelled secondary antibodies indicate that these antibodies bind specifically to the granules of mast cells in cytospin preparations of enzymically dispersed lung, tonsil, colon and skin. Under the same conditions, there is no binding to peripheral blood basophils purified on a Percoll gradient. The monoclonal antibodies are effective also at identifying mast cells in paraffin-embedded tissue.
An antigen-capture ELISA procedure was developed in which the IgG fraction of the polyclonal anti-tryptase was adsorbed to polystyrene microtitre plates, followed by the addition of tryptase standard or sample, and then the monoclonal anti-tryptase AAS. The signal was amplified using biotinylated goat antibody specific for mouse IgG (Amersham) and avidin–biotin–peroxidase complex (Dako), and the colour reaction of the substrate o-phenylenediamine dihydrochloride (Sigma) was measured at 490 nm. The assay was capable of measuring tryptase at concentrations as low as 1 ng/ml (Fig. 1a).

Tryptase levels were investigated in bronchoalveolar lavage (BAL) fluid recovered from normal subjects and patients with bronchial carcinoma, extrinsic allergic alveolitis and sarcoidosis. BAL fluid was obtained by fibreoptic bronchoscopy with instillation of two 60 ml aliquots of saline. There were clear differences between disease groups, with highest tryptase concentrations in the BAL fluid of patients with interstitial disease (Fig. 1b). Tryptase levels were closely correlated with mast cell numbers as enumerated by Toluidine Blue dye binding of cytospin preparations (P < 0.0005, n = 22, Spearman correlation).

Recent reports indicate an elevation in tryptase concentration in the BAL fluid of mild asthmatics after allergen instillation [6], in skin chamber fluid after cutaneous allergen challenge [7, 8] and in the serum of patients with systemic mastocytosis and anaphylaxis [9]. Tryptase should be a useful biochemical marker of mast cell activation.

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Fig. 1. Molecular species of term human lung PC

Abbreviation used: PC, phosphatidylcholine.