Granulocyte Recruitment by Human Mast Cell Tryptase

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Tryptase is a tetrameric serine protease which is stored almost exclusively in the secretory granules of mast cells. Substantial quantities of this enzyme are released as a consequence of mast cell activation in allergic disease. The pathobiological role of tryptase is not well understood, though this enzyme can cleave a number of potential substrates [reviewed in ref. 1]. Tryptase can efficiently degrade certain regulatory peptides including the bronchodilator, vasoactive intestinal peptide and the potent vasodilator cal-citonin-gene-related peptide; it may have a role in kinin generation, and by activating stromelysin, it may participate in processes of tissue degradation, and it can act as a growth factor for fibroblasts.

Microvascular leakage has been observed in the skin of guinea pigs following injection of purified human mast cell tryptase. As we have found that the increase in vascular permeability can be inhibited by antihistamine pretreatment, and that tryptase can stimulate histamine release from guinea pig mast cells in vitro, it would seem likely that the effect is mediated by tryptase-induced mast cell activation. Injection of tryptase into guinea pig skin or the mouse peritoneum results, within 6 h, in a neutrophil-rich inflammatory infiltrate. We have investigated potential mechanisms of human granulocyte recruitment by tryptase using in vitro models.

In modified Boyden chambers with neutrophils purified from peripheral blood on Lymphoprep, we have found that tryptase can itself act as a chemoattractant even at concentrations of 6 mU/ml (where 1 mU is defined as that amount which can hydrolyse 1 nmol of the peptide substrate N- α -benzoyl-DI-arginine /?-nitroanilide per minute at 25 °C). This action was inhibited by protease inhibitors including leupeptin, and by heat treatment, indicating dependence on an active catalytic site. Incubation of purified neutrophils with tryptase was associated with a shape change reaction within minutes, as revealed by scanning electron microscopy.

Eosinophils purified from human peripheral blood (using a Percoll gradient followed by a negative immunomagnetic separation procedure with a magnetic cell sorter system), also exhibited chemotaxis and shape change responses with tryptase. Chemotaxis was observed at tryptase concentrations as low as 2.5 mU/ml, but was optimal at 10 mU/ml, and was inhibited by protease inhibitors and by heat treatment of the tryptase. In separate experiments, incubation of purified eosinophils with tryptase stimulated the dose-dependent secretion of eosinophil cationic protein into supernatants, as detected by specific fluoroimmuno-assay. This effect was most pronounced at concentrations of tryptase greater than those optimum for chemotaxis, and

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Fig. 1. Cellular targets of human mast cell tryptase.

also required tryptase to be catalytically active. A parallel increase in lactate dehydrogenase was not observed, suggesting that the effect was non-cytotoxic.

Incubation of purified tryptase with the human epithelial cell line H292 stimulated DNA synthesis as assessed by ³H-thymidine incorporation. This was maximal after 24 h at a concentration of 25 mU/ml tryptase and could be inhibited with protease inhibitors. FACS analysis revealed that tryptase induced the up-regulation of ICAM-1 expression on these cells, while enhanced release of interleukin-8 (IL-8) was detected by immunoassay.

These studies indicate that human mast cell tryptase can profoundly alter cell behaviour (fig. 1). This protease may act as an important mediator of granulocyte recruitment in allergic disease, acting directly on granulocytes, up-regulating expression of adhesion molecules and inducing release of the potent granulocyte chemoattractant IL-8.

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References

Walls AF: The roles of neutral proteases in asthma and rhinitis; in Busse WW, Holgate ST (eds): *Asthma and Rhinitis*. Boston, Blackwell, 1995, pp 801-825.