Inhibitors of Chymase as Mast Cell-Stabilizing Agents: Contribution of Chymase in the Activation of Human Mast Cells

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ABSTRACT

There has long been evidence that inhibitors of chymotryptic proteinases can inhibit the degranulation of rodent mast cells, but their actions on human mast cells and the contribution of mast cell chymase itself have received little attention. We investigated the ability of the selective chymase inhibitor Z-Ile-Glu-Pro-Phe-CO2Me and other proteinase inhibitors to inhibit chymase and cathepsin G activity, and we examined their potential to modulate the responsiveness of mast cells dispersed from human skin, lung, and tonsil tissues. IgE-dependent histamine release from skin mast cells was inhibited by up to about 80% after preincubation with Z-Ile-Glu-Pro-Phe-CO2Me (up to 0.1 μM), 70% with chymostatin (17 μM), and 60% with soybean trypsin inhibitor (0.5 μM). The mast cell-stabilizing properties of chymase inhibitors appeared to be greater for skin mast cells than for those from lung, whereas tonsil mast cells were relatively unresponsive. There were marked differences in the time course of responses to inhibitors, and the effect was dependent on the stimulus, with calcium ionophore-induced histamine release being unaffected. Incubation of dispersed skin, lung, or tonsil cells for up to 45 min with purified chymase failed to induce histamine release, although preincubation of cells with chymase was able to suppress IgE-dependent activation. Chymase could thus contribute to mast cell degranulation and after secretion could provide a feedback mechanism to limit this process. Nevertheless, inhibitors of chymase can be potent mast cell stabilizers, particularly in the skin.

Proteases represent the most abundant products of human mast cells, and a cumulative total of up to 60 pg may be stored in the secretory granules of each cell. Prominent among these are the tryptic enzyme tryptase, the chymotryptic enzymes chymase and cathepsin G, and a carboxypeptidase. These proteases are emerging as important mediators and as potential targets for therapeutic intervention in allergic disease. After their secretion from activated mast cells, there is evidence that they may have major roles in processes of inflammation, tissue remodeling, bronchoconstriction, and mucus secretion (Walls, 1998). The proteases have the potential to alter the behavior of mast cell themselves. Recently, we found that tryptase can itself stimulate the activation of human (He et al., 1998) and rodent (He and Walls, 1997) mast cells, and this property may be responsible for the microvascular leakage (Molinari et al., 1995; He and Walls, 1997) and bronchoconstriction (Molinari et al., 1996) that have been observed in vivo after transfer of this protease into animal models. The potential for tryptase-induced mast cell activation to represent an amplification mechanism in allergic inflammation raises the possibility that other mast cell proteases alone or in combination could alter mast cell function after their release. A rat chymase has been reported to induce the activation of rat serosal mast cells (Schick et al., 1984; Schick, 1990), suggesting that this protease also may trigger further mast cell activation after its release. However, the actions of the human counterpart on human mast cells have not been investigated.

There have long been suggestions that a chymotryptic protease may be involved in processes of anaphylactic mast cell degranulation. Austen and Brocklehurst (1960) observed that allergen-induced histamine release could be inhibited using various inhibitors and synthetic substrates of chymotryptic enzymes. In subsequent studies, it has been noted that the activation of purified rat peritoneal mast cells can be inhibited using various inhibitors and synthetic substrates of chymotryptic enzymes. In subsequent studies, it has been noted that the activation of purified rat peritoneal mast cells can be inhibited using various inhibitors and synthetic substrates of chymotryptic enzymes.
and a Bowman-Birk soybean protease inhibitor (all of which are inhibitors of chymase) and by using a neutralizing antibody specific for the rat chymase known as rat mast cell protease 1 and synthetic substrates for chymotryptic proteases, including \(N\)-succinyl-l-Ala-l-Ala-l-Pro-l-Phe-thio-benzyl ester (AAPF-S-Bzl) and \(N\)-succinyl-Phe-Pro-Phe-p-nitroanilide (Ishizaka and Ishizaka, 1984; Kido et al., 1988; Emadi-Khiva and Pearce, 1998). These findings have prompted the idea that chymase could have a key role in mast cell activation, but the appropriate studies with human mast cell populations have not been performed. Certain broad-spectrum protease inhibitors have been found to inhibit histamine release from some human tissues (Hultschi et al., 1988; Dietze et al., 1990; Yanagida et al., 1997), but this has not been tested with potent or selective inhibitors of chymotryptic proteases. In the present study, we examined the actions of the selective chymase inhibitor Z-Ile-Glu-Pro-Phe-CO\(_2\) Me (ZIGPFM) (Bastos et al., 1995) and other inhibitors of chymotryptic enzymes, as well as of human mast cell chymase itself, on the function of mast cells dispersed from various sources of human tissues.

## Materials and Methods

### Reagents

Soybean trypsin inhibitor (SBTI), chymostatin, aprotinin, nitroanilide (NA) substrates [\(N\)-benzoyl-DL-arginine-p-NA (BAPNA), \(N\)-succinyl-l-Ala-l-Ala-l-Ala-p-NA, \(N\)-succinyl-l-Ala-l-Ala-l-Pro-l-Phe-p-NA (AAPFpNA)], 5,5'-dithiobis(2-nitrobenzoic acid), histamine dihydrochloride, collagenase (type I), hyaluronidase (type I), BSA (fraction V), penicillin and streptomycin, minimum essential medium containing 25 mM HEPES, heparin agarose, Sephacryl S-200, calcium ionophore A23187, Tris base, and kindly provided by Ferring Research Institute (Southampton, UK). HEPES and all other chemicals were of Seikagaku (Tokyo, Japan). AAPF-S-Bzl was obtained from Bachem Toxicolor System for the assay of endotoxin was purchased from Toxicolor Systems, (Saffron Walden, Essex, UK). HEPES and all other chemicals were of analytical grade and were obtained from BDH (Poole, Dorset, UK). Goat anti-human IgE (inactivated) was purchased from Serotec (Kidlington, Oxford, UK). FCS from obtained Life Technologies, Inc. (Paisley, Renfrewshire, UK). α-Phthalaldehydyledehyde was obtained from Fluka (Gillingham, Dorset, UK). Coomassie protein assay reagent was purchased from Pierce (Rockford, IL). Silver staining kit was obtained from BioRad (Hemel Hempstead, UK). The Toxicolor System for the assay of endotoxin was purchased from Seikagaku (Tokyo, Japan). AAPF-S-Bzl was obtained from Bachem (Saffron Walden, Essex, UK). HEPES and all other chemicals were of analytical grade and were obtained from BDH (Poole, Dorset, UK). The chymase inhibitor ZIGPFM (Bastos et al., 1995) was synthesized and kindly provided by Ferring Research Institute (Southampton, UK).

### Characterization of Proteinase Inhibitors

To establish inhibitor concentration-response curves, inhibitors were incubated at five times the indicated concentration with either 0.9 \(\mu\)g/ml human skin chymase, 0.5 \(\mu\)g/ml human neutrophil cathepsin G, or 0.5 \(\mu\)g/ml bovine chymotrypsin for 30 min on ice in 120 mM NaCl and 50 mM Tris · HCl, pH 7.6. For assay, samples were diluted 5-fold with substrate solution to give a final concentration of 0.7 mM AAPFpNA (for chymase and chymotrypsin) or 0.5 mM AAPF-S-Bzl and 0.1 mM 5,5'-dithiobis(2-nitrobenzoic acid) (for cathepsin G) at 25°C. To determine the inhibition constants for chymostatin, SBTI, and ZIGPFM with chymase, assays were conducted in the same buffer as above, in the presence of various concentrations of inhibitor, and with AAPFpNA concentrations ranging from 0.4 to 8.0 mM. \(K_{i,\text{app}}\) and \(V_{\text{max,app}}\) values were calculated by iterative nonlinear least-squares regression of the Michaelis-Menten equation using the SPSS statistical software package. \(K_{i,\text{app}}\) values were calculated from secondary plots of \(K_{i,\text{app}}/V_{\text{max,app}}\) versus [I], and \(K_{i,\text{app}}^\prime\) values were calculated from secondary plots of 1/[\(V_{\text{max,app}}\)] versus [I].

### Mast Cell Disruption and Challenge

Human lung, tonsil, and skin tissue were obtained at lobectomy, tonsillectomy, and circumcision, respectively, and were dispersed and challenged with the use of procedures described previously (He et al., 1998). After chopping finely with scissors, tissue fragments were digested with 1.5 mg/ml collagenase and 0.75 mg/ml hyaluronidase in minimum essential medium containing 2% FCS, 200 U/ml penicillin, and 200 \(\mu\)g/ml streptomycin (1 g tonsil/10 ml buffer for 60 min, 1 g lung/10 ml for 65 min, and 1 g skin/15 ml for 75 min) at 37°C. Dispersed cells were passed through nylon gauze (100-μm mesh), washed, and maintained in the medium at room temperature on a mechanical roller overnight. Mast cell numbers were determined by light microscopy after staining according to the procedure of Kimura (He et al., 1998). In preparations of dispersed skin, lung, or tonsil cells, mast cells comprised 5.2 ± 1.0 (n = 12), 4.2 ± 0.9 (n = 14), or 0.5 ± 0.06% (n = 14), respectively.

After warming at 37°C for 5 min, an aliquot of 100 \(\mu\)l of cell suspension (containing 4–6 × 10\(^5\) mast cells) was added to 50 \(\mu\)l of purified chymase, control secretagogue, or inhibitor in HEPES-buffered salt solution (HBSS, pH 7.4) with 1.8 mM CaCl\(_2\) and 0.5 mM MgCl\(_2\) (complete HBSS) and incubated for up to 45 min at 37°C. In some tubes, cells were incubated with 25 \(\mu\)l of inhibitor or chymase for up to 30 min before the addition of 25 \(\mu\)l of stimulus. The reaction was terminated by adding 150 \(\mu\)l of ice-cold HBSS, and the tubes were centrifuged immediately (500g, 10 min, 4°C).

Preliminary experiments involving the incubation of tissues with control stimuli over a range of concentrations indicated that antibody specific for IgE at a concentration of 1% or calcium ionophore A23187 at 1 \(\mu\)M could provoke maximal histamine release without evidence of cytotoxicity (i.e., histamine release was inhibited using the metabolic inhibitors 2-deoxy-o-glucose and antimycin A; He et al., 1998). For this reason, these secretagogues were used at these standard concentrations throughout. All experiments were performed in duplicate. For the measurement of total cellular histamine concentration, the cell suspension in certain tubes was boiled for 6 min. Histamine concentrations in all supernatants were determined using a glass fiber-based, fluorometric assay (Lundbeck Diagnostics, Copenhagen, Denmark) as described previously (Nolte et al., 1987; He et al., 1998). Histamine release was expressed as a percentage of total cellular histamine levels and adjusted to take into account spontaneous release measured in tubes in which cells had been incubated with the HBSS diluent alone. Calculations of net inhibition of histamine release took into account the amount of histamine release in the presence of the inhibitor but in the absence of stimulus.

### Purification and Characterization of Chymase

The method of purification of chymase from human skin has been reported previously (He and Walls, 1998). In brief, chymase activity was extracted from homogenized human skin tissue with a high salt buffer and then subjected to heparin agarose column chromatography. The chymase-rich fractions were applied to a Sephacryl S-200 column, and the purified chymase was concentrated with C-10 Centricon centrifugal concentrators (Amicon, Stonehouse, Gloucestershire, UK) before storage at −80°C in 1 M NaCl. Chymase activity in column fractions and in the purified preparation was determined by measuring the hydrolysis of 0.7 mM AAPFpNA (Schechter et al., 1988; McEuen et al., 1995). The protein concentration was determined using the Coomassie blue dye binding procedure according to the manufacturer’s protocol with BSA as standard.

The specific activity of the chymase prepared was 4.9 U/mg, where 1 U of enzyme activity was taken as the amount that catalyzed the cleavage of 1 \(\mu\)mol of AAPFpNA/min at 25°C. Analysis by SDS-polyacrylamide gel electrophoresis with silver staining revealed a single diffuse band with an apparent molecular mass of approximately 30 kDa, and the identity as chymase was confirmed by Western blotting with rabbit antiserum specific for human chymase (McEuen et al., 1998a). Using the Toxicolor System, endotoxin was found to be present at a concentration of less than 41 pg/mg chymase. No contamination with trypsinic activity was detected using the substrate 20 mM BAPNA in 0.1 M Tris · HCl and 1 M glycerol, pH 8.0.
containing 1 mg/ml BSA (Smith et al., 1984) or with elastolytic activity using 1.4 mM N-succinyl-t-Ala-t-Ala-t-Ala-p-NA in the same buffer as used with BAPNA (Nakajima et al., 1979). Immediately before its addition to cells, chymase was diluted first with sterile distilled water, adjusting the NaCl concentration to 0.15 M, and then with normal saline to obtain the required chymase concentration.

Statistical Analysis. All statistical analyses were performed using StatView software (Version 4.02; Abacus Concepts, Berkeley, CA). Data are shown as the mean ± S.E. for the number of experiments indicated. Where ANOVA indicated significant differences between groups, for the preplanned comparisons of interest, paired Student’s t tests were applied. For all analyses, p < .05 was taken as statistically significant.

Results

Properties of Inhibitors. On the basis of IC$_{50}$ determinations, ZIGPFM and chymostatin were found to be more than 20 times more potent as inhibitors of chymase than of cathepsin G activity toward the synthetic substrate (Table 1). SBTI, on the other hand, was about 6-fold more potent in inhibiting cathepsin G than chymase, although it was more effective as a chymase inhibitor than chymostatin. Aprotinin had no inhibitory actions on chymase activity, but it did inhibit cathepsin G. For purposes of comparison, IC$_{50}$ values also were calculated for the nonmast cell proteinase chymotrypsin (Table 1).

Chymostatin acted as a competitive inhibitor of chymase, whereas SBTI and ZIGPFM both exhibited a mixed pattern of inhibition. Where appropriate, values of $K_i$ and $K'_i$ were determined (Table 2). The concentrations at which chymase inhibitors were used in the studies with mast cells were determined on the basis of the $K_i$ or $K'_i$ values, and the concentrations of aprotinin applied were based on its IC$_{50}$ value with cathepsin G.

Effect of Chymase Inhibitors on Histamine Release. Preincubation of dispersed skin cells with various doses of the most selective chymase inhibitor ZIGPFM for 5 min before challenge with anti-IgE resulted in a dose-dependent inhibition of histamine release (Fig. 1). Histamine release was inhibited approximately 80% by 5 min preincubation with 100 nM ZIGPFM, although no significant inhibition of histamine release was observed with either a 30-min preincubation period or with no preincubation. ZIGPFM did not significantly alter IgE-dependent histamine release from lung or tonsil cells after preincubation periods of up to 30 min (data not shown). At concentrations up to 1000 nM, ZIGPFM did not by itself induce histamine release from lung, tonsil, or skin cells (data not shown).

Table 1

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC$_{50}$ Values</th>
<th>nM</th>
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<tr>
<td></td>
<td>Chymase</td>
<td>Cathepsin G</td>
</tr>
<tr>
<td>ZIGPFM</td>
<td>42</td>
<td>920</td>
</tr>
<tr>
<td>Chymostatin</td>
<td>420</td>
<td>12,000</td>
</tr>
<tr>
<td>SBTI</td>
<td>125</td>
<td>20</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>N.I.</td>
<td>1,100</td>
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N.I., no inhibition.

The broad-spectrum inhibitor of chymotryptic proteinase, chymostatin, was effective at inhibiting IgE-dependent histamine release from both skin and lung cells preincubated for periods of 0 to 30 min (Fig. 2). At a concentration of 10 µg/ml (17 µM), it was able to inhibit histamine release from skin or lung cells by approximately 70 or 80%, respectively, although significant inhibition of histamine release was not observed from tonsil cells under the same conditions. However, 10 µg/ml chymostatin was by itself able to stimulate histamine release (15 ± 5.4%) from lung cells but had no such effect on skin and tonsil cells (data not shown). SBTI at a concentration of 10 µg/ml (0.5 µM) was able to inhibit IgE-dependent histamine release from skin cells by up to about 60% (Fig. 2). For none of these inhibitors was there any association between the degree of inhibition seen and the magnitude of the control response. Aprotinin at up to 100 µg/ml (15 µM) did not have any apparent effect on anti-IgE-induced histamine release from either skin, lung, or tonsil cells, although there was a trend for inhibition of that from skin (Fig. 2). Neither SBTI- nor aprotinin-induced histamine release from any of the sources of cells at the concentrations tested (data not shown).

ZIGPFM, chymostatin, SBTI, and aprotinin were tested for their ability to modulate histamine release induced by calcium ionophore from skin, lung, and tonsil mast cells. The concentrations of inhibitor and the preincubation periods with cells were the same as those used in the parallel studies of IgE-dependent histamine release. Although there was a

### Table 2

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$K_i$ (nM)</th>
<th>$K'_i$ (nM)</th>
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<tbody>
<tr>
<td>ZIGPFM</td>
<td>15 ± 5.0</td>
<td>63 ± 21</td>
</tr>
<tr>
<td>Chymostatin</td>
<td>94 ± 13</td>
<td></td>
</tr>
<tr>
<td>SBTI</td>
<td>98 ± 34</td>
<td>132 ± 37</td>
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Calculated values are expressed ± S.E. of the linear regression.

Fig. 1. The effects of ZIGPFM on anti-IgE-induced histamine release from dispersed skin cells. The cells were preincubated with various concentrations of the inhibitor or with buffer alone for 5 min at 37°C before challenge with the stimulus. Mean ± S.E. values are shown for five separate experiments.

![Image](path/to/image)
trend for chymostatin to inhibit the ionophore-induced histamine release from skin and lung mast cells, significant inhibition of histamine release was not achieved with any of the inhibitors (data not shown).

**Effect of Chymase on Mast Cells.** To examine the possibility that the inhibition of IgE-dependent histamine release by chymase inhibitors could be related to the inhibition of endogenous chymase, the ability of purified chymase to induce histamine release was investigated. However, incubation of dispersed skin, lung, or tonsil cells for periods up to 45 min with chymase at concentrations from 0.03 to 30 mU/ml (6.1 ng/ml to 6.1 μg/ml) failed to induce significant histamine release (Fig. 3A). In the same cell preparations, anti-IgE and calcium ionophore were effective in provoking histamine release (Fig. 3B). In the same cell preparations, anti-IgE and calcium ionophore were effective in provoking histamine release (Fig. 3B).

Incubation of skin, lung, or tonsil cells with chymase at concentrations of 0.1 or 1 mU/ml for periods of 0, 5, or 30 min had no effect on the degree of histamine release achieved on subsequent anti-IgE challenge (data not shown). However, the treatment of skin cells with 10 mU/ml chymase was associated with an apparent reduction in IgE-dependent histamine release of approximately 60% (Fig. 4). Similar findings were observed with tonsil cells after 5-min preincubation, although not with the other preincubation periods tested; no consistent effects with lung cells were noted. Under the same conditions as tested with IgE-dependent histamine release, preincubation of cells with 0.1 to 10 mU/ml chymase for 0, 5, or 30 min failed to alter the amount of histamine release on subsequent challenge with calcium ionophore (data not shown).

**Discussion**

Our findings indicate that inhibitors of chymotryptic proteases may effectively inhibit IgE-dependent activation of certain populations of human mast cells. The inhibitors with greatest potency and selectivity for chymase were more effective as mast cell stabilizers than were those for cathepsin G, the other major chymotryptic enzyme in human mast cells. In contrast to previous reports with a rat chymase, however, exogenous human chymase failed to provoke mast cell degranulation. Our findings highlight substantial differences in mast cell responsiveness between species and between mast cell populations dispersed from different tissues in humans.

With the selective inhibitor of chymase ZIGPFM, histamine release from dispersed lung or skin cells in response to chymase, anti-IgE, or calcium ionophore. A, chymase-induced histamine release after incubation for 15 ( ), 20 ( ), or 45 ( ) min at 37°C. B, anti-IgE- or calcium ionophore-induced histamine release after 15-min incubation at 37°C. Mean ± S.E. values are shown for 5 to 8 separate experiments with chymase and 8 (skin) or 12 (lung) with anti-IgE or with calcium ionophore. *p < .05 in comparison with the baseline values (paired Student’s t test). Mean ± S.E. spontaneous histamine release was 8.4 ± 1.0% with lung cells and 7.2 ± 1.0% with skin cells.
mine release from skin mast cells was inhibited by up to 80% with a concentration of as little as 0.1 μM. Moreover, with chymostatin, which is also a potent inhibitor of chymase, inhibition of 70 or 80% from skin and lung cells was achieved with a concentration of 17 μM; with 0.5 μM SBTI, there was about 60% inhibition of histamine release from skin cells. The degree of inhibition observed was of a similar order as that achieved with tryptase inhibitor APC366 and with certain other inhibitors of tryptic proteinases (He et al., 1998), but it was very high compared with that found with antiallergic drugs with mast cell-stabilizing properties. For example, the β2-adrenoceptor agonist salbutamol has, at 1 μM, been reported to inhibit IgE-dependent histamine release from skin, lung, and tonsil cells by about 20%, whereas cromoglycate at concentrations up to 1000 μM failed to inhibit histamine release from skin cells and inhibits histamine release from both lung and tonsil cells by just 12% (Okayama and Church, 1992). Such comparisons must indicate the importance of proteolytic mechanisms in processes of mast cell activation and call attention to the potential value of protease inhibitors as mast cell-stabilizing agents.

As has been noted with various antiallergic drugs (Church et al., 1997), cells dispersed from different tissues exhibited marked differences in the extent to which they could be stabilized by chymase inhibitors. There also were differences in the time course of responses to these inhibitors, although compound stability may have influenced pharmacological actions. It seems likely that mast cell responsiveness to the various proteinase inhibitors will reflect differences in proteinase composition. Through immunocytochemistry with antibodies specific for tryptase and chymase, subsets of mast cells have been categorized according to whether they contain tryptase and chymase (MC1), or tryptase but not chymase (MC2; Irani et al., 1986; Buckley et al., 1999). Cathepsin G (Schechter et al., 1990) and carboxypeptidase (Irani et al., 1991) have also been localized selectively to mast cells of the MC2 phenotype. The MC1 subpopulation is generally most abundant in connective tissues, and the MC2 subset is most abundant in mucosal tissues. More than 99% of skin mast cells have been found to contain chymase, but the corresponding figure for dispersed lung preparations is approximately 10% (Irani et al., 1989). The relative numbers of each mast cell phenotype have not been investigated, but our findings do raise the possibility that there may be some association between the presence of chymase in mast cell subpopulations and the degree to which histamine release can be inhibited by chymase inhibitors.

The failure of exogenous purified human chymase to stimulate the release of histamine from any of the three sources of human mast cells may appear surprising, although the observations are in keeping with the finding that microvascular leakage induced in the skin of guinea pigs by the injection of human chymase is unaffected by antihistamine pretreatment of the animals (He and Walls, 1998). The release of human chymase, unlike tryptase (He et al., 1998), therefore does not appear to provide an amplification signal that triggers further mast cell degranulation. The studies with chymase inhibitors nevertheless do suggest that chymase may play a key role in the activation of human mast cells. The nature of the substrate cleaved and its precise location remain open to conjecture. Experimental evidence has been presented that rat chymase may cleave a 90-kDa membrane component during IgE-dependent activation (Schick, 1990), as well as being able to generate a histamine-releasing peptide from albumin (Cochrane et al., 1993). It would seem likely, however, that the inhibitors used in the present study have inhibited the cleavage of a substrate sequestered within the cell or exposed only once the process of mast cell degranulation has commenced. In this respect, it is of interest that IgE-dependent activation of permeabilized rat peritoneal mast cells loaded with a fluorescent substrate has been found to be associated with an increase in chymotryptic activity within the cell (Emadi-Khiav and Pearce, 1998). The extent to which the various inhibitors used in the present study may have penetrated the cells and acted intracellularly is unclear; however, mast cells do have the capacity to endocytose certain extracellular proteins, including eosinophil peroxidase (Dvorak et al., 1985) and major basic protein (Butterfield et al., 1990), and Kido et al. (1988) reported that SBTI and even F(ab’)2 fragments of a rat chymase-specific antibody may be taken up into the granules of rat peritoneal mast cells after a brief incubation period.

Chymase is stored in mast cell secretory granules in a form that is catalytically active (Harvima et al., 1993), although at pH 5.5, the reported pH of the mast cell granule, chymase has relatively little activity toward synthetic substrates (McEuen et al., 1995). It does seem likely, however, that the conditions within the secretory granules will become more favorable for chymase activity after the initiation of degranulation, a process that at the ultrastructural level in human mast cells involves loss of the crystalline structure of granules, the apparent solubilization of granule contents, the fusion of granule membranes with one another and with the plasma membrane, and the formation of complex degranulation channels that open into the extracellular space (Caulfield et al., 1990). Emadi-Khiav and Pearce (1998) recently suggested...
that at least in rat mast cells, degranulation may be associated with the activation of a zymogen leading to an increase in chymotryptic activity within the cell. The activation of human chymase with dipeptidyl peptidase 1, however, occurs with a pH optimum in the neutral range (McEuen et al., 1998a), an observation that appears to indicate that this process is restricted to the early stages of vesicle formation and before the incorporation of chymase into mature granules.

Our observation that the chymase inhibitors failed to inhibit calcium ionophore-induced histamine release from human mast cells is consistent with findings reported elsewhere with chymotatin and certain other inhibitors and substrates of chymotryptic activity (Emadi-Khivai and Pearce, 1998) and suggest that the requirement for chymotryptic activity is restricted to IgE-dependent cell activation. However, the broad-spectrum inhibitors 1-tosylamide-2-phenylethyl chloride and diisopropylfluorophosphate have been reported to inhibit histamine release from human mast cells in response to stimulation with calcium ionophore (Hultsch et al., 1988; Yanagida et al., 1997), suggesting that a protease other than chymase could be involved in cell activation with this stimulus.

The relative selectivity of ZIGPFM and chymostatin for chymase must call particular attention to this abundant mast cell chymotryptic enzyme as the primary target of these inhibitors in the modulation of IgE-dependent mast cell degranulation. Similarly, the inhibitor profile of the proteinase inhibitors used would lead one to discount cathepsin G as having a major role in mediating degranulation. Nevertheless, one cannot exclude the possibility that there may be a chymotryptic protease other than chymase in mast cells that may be pivotal in IgE-dependent degranulation. Alternatively, there may be new forms of chymase yet to be identified that could have a role. Multiple cDNA sequences have been derived for rat and mouse chymases, and the corresponding proteases exhibit important differences in their enzymatic actions and in their distribution in mast cell populations (Walls, 1995). Just one full-length sequence has been reported for the human enzyme to date, but high salt extracts of human skin, heart, lung, and other tissues do contain at least two distinct chymases that differ in affinity for heparin and in the relative quantities in different tissues (McEuen et al., 1998b), and the potential for a distinct human mucosal mast cell chymase is suggested by the detection of immunoreactive chymase (Beil et al., 1997) and chymotryptic activity (Huntley et al., 1985) in mast cells in this tissue compartment.

The observation that incubation of cells with a preparation of purified chymase could result in a reduction in histamine release on stimulation with anti-IgE antibody was unexpected given the failure of chymase alone to stimulate histamine release. Antigen-induced desensitization has been reported to involve a proteinase that can be inhibited by diisopropylfluorophosphate, but the precise mechanism remains to be elucidated (Ishizaka et al., 1985). Further investigations will be required to explore whether chymase can cleave an extracellular domain of the FceRI receptor or even a portion of the IgE molecule, thereby reducing the extent of cell activation with anti-IgE.

The results of the present study suggest that chymase or a chymotryptic protease with a similar inhibitor profile can act on an intracellular substrate to mediate IgE-dependent activation of human mast cells. Paradoxically, chymase released after mast cell degranulation could also provide a feedback mechanism, restricting further degranulation. However, inhibitors of chymase can be potent stabilizers of human mast cells and particularly those in skin tissue. It is possible that the development of potent and selective chymase inhibitors will lead to useful new drugs for the treatment of mast cell-mediated inflammatory conditions.

References


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