Neutrophil Defensins Induce Histamine Secretion from Mast Cells: Mechanisms of Action

A. Dean Befus, Connie Mowat, Mark Gilchrist, Jing Hu, Samuel Solomon and Andrew Bateman

*J Immunol* 1999; 163:947-953;
http://www.jimmunol.org/content/163/2/947

**References** This article cites 63 articles, 32 of which you can access for free at:
http://www.jimmunol.org/content/163/2/947.full#ref-list-1

**Subscription** Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Neutrophil Defensins Induce Histamine Secretion from Mast Cells: Mechanisms of Action

A. Dean Befus, Connie Mowat, Mark Gilchrist, Jing Hu, Samuel Solomon, and Andrew Bateman

Defensins are endogenous antimicrobial peptides stored in neutrophil granules. Here we report that a panel of defensins from human, rat, guinea pig, and rabbit neutrophils all have histamine-releasing activity, degranulating rat peritoneal mast cells with EC50 ranging from 70 to 2500 nM, and between 45 and 60% of the total histamine released. The EC50 for defensin-induced histamine secretion correlates with their net basic charge at neutral pH. There is no correlation between histamine release and antimicrobial potency. Degranulation induced by defensins has characteristics similar to those of activation by substance P. The maximum percent histamine release is achieved in <10 s, and it can be markedly inhibited by pertussis toxin (100 ng/ml) and by pretreatment of mast cells with neuraminidase. These properties differ from those for degranulation induced by IgE-dependent Ag stimulation and by the calcium ionophore A23187. GTPase activity, a measure of G protein activation, was induced in a membrane fraction from mast cells following treatment with defensin. Thus, neutrophil defensins are potent mast cell secretagogues that act in a manner similar to substance P and 48/80, through a rapid G protein-dependent response that is mechanistically distinct from Ag/IgE-dependent mast cell activation. Defensins may provide important pathways for communication between neutrophils and mast cells in defenses against microbial agents and in acute inflammatory responses. The Journal of Immunology, 1999, 163: 947–953.

In studies largely with human basophils, a diverse group of substances that induce histamine secretion has been identified. These are collectively referred to as histamine-releasing factors (HRF) and can be derived from T and B lymphocytes, alveolar macrophages, platelets, mononuclear cells, fibroblasts, and neutrophils. HRF have been found in nasal washings from atopic individuals (15), bronchoalveolar lavage fluids from individuals with pulmonary fibrosis (16), tissue fluids from Ag-stimulated late phase skin reactions (17), and PBMC of individuals with occupational asthma (18). Several forms of HRF can be derived from mononuclear cells that have been activated (14). An 8- to 10-kDa form appears to be connective tissue-activating peptide, and its closely related cleavage product is neutrophil-activating peptide (19). Monocyte chemotactic and activating factor (20), monocyte chemotactic protein 1 (21), macrophage inflammatory protein-1α (22), RANTES (23), macrophage chemotactic protein-2 and -3 and fibroblast-induced cytokine (24), and IL-3 (25) also induce histamine secretion from human basophils. In addition, a novel IgE-dependent HRF has been recently defined by MacDonald and colleagues as highly homologous with mouse p21 and human p23 (26). Neutrophil-derived HRF appears to be different from these, because it is constitutively produced, heat stable, and has an estimated molecular mass of 1400–2300 Da (27). Others have identified that HRF activity from neutrophils lies within cationic lysozyme proteins of about 5 kDa (28).

Recently, there has been an explosion of interest in defensins, a family of highly cationic proteins of about 3500 Da derived from neutrophils and other cells (29–31). Defensins are variably cationic, arginine-rich, nonglycosylated peptides that contain a characteristic cysteine motif. They are best known for their antimicrobial activity. Defensins have also been identified as corticotatins because they bind specifically to the ACTH receptor and inhibit...
the actions of ACTH on the adrenal glands (32, 33). They are also chemotactic for human monocytes (34), and at least two guinea pig defensins can induce histamine release from rat peritoneal mast cells (PMC) (35). Given this single report about the ability of defensins to induce histamine secretion and the possibility that neutrophil-derived HRF activity involves defensins (27, 28), we characterized the ability of several defensins to induce histamine secretion and studied their mode of action.

Materials and Methods

Animals

Outbred male Sprague Dawley rats (375–450 g) purchased from Charles River Canada (St. Constant, Canada) were used. They were maintained in an isolated room in filter-top cages to minimize unwanted infections. The animals were given food and water ad libitum and were maintained on a 12-h light (0700 h), 12-h dark (1900 h) cycle. No rats were used for experimentation until a minimum of 1 wk following arrival in our facilities, so as to reduce the effects of stress associated with transport, handling, and new environmental conditions. All experimental procedures were approved by the University animal care committee and were performed in accordance with the guidelines of the Canadian Council on Animal Care.

Either normal rats or rats infected 35–100 days previously with 3000 third stage larvae of the nematode, *Nippostrongylus brasiliensis* (to induce mast cell hyperplasia and IgE-dependent sensitization) were used to isolate PMC for study (8).

Mast cell isolation and enrichment

Normal rats or rats previously infected with *N. brasiliensis* were anesthetized, and 15 ml of cold Tyrode’s solution containing 12 mM HEPES and 1% BSA, pH 7.3 (HTB), were injected into the peritoneal cavity. The abdomen was massaged, and the fluid was collected and centrifuged for 4 min at 180 × g at 4°C. Peritoneal exudate cells were layered on a 30%/80% discontinuous gradient of Percoll (Pharmacia, Dorval, Canada) for 20 min at 220 × g at 4°C. The cell pellet was washed twice and examined for mast cell purity (≥98%) and viability (≥97%) by trypan blue exclusion (8). Mast cell viability was also assessed using trypan blue exclusion following treatment with each of the peptides tested as well as with pertussis toxin. None of the treatments significantly altered cell purity (98%) and viability (97%) by trypan blue exclusion (8).

Histamine secretion and its modulation by pertussis toxin and neuraminidase

For studies of histamine secretion, 50,000 highly enriched PMC (≥98%) and the secretagogues to be used were preincubated separately at 37°C for 5 min, and then the secretagogues were added to the cells and incubated at 37°C for 10 min. The reaction was terminated by the addition of 1 ml of cold HTB and was centrifuged at 4°C to separate the supernatant and cell pellet. After the pellets were brought to 1.5 ml using HTB, the samples were boiled for 10 min to release cell-associated histamine and destroy histaminase activity. After TCA precipitation of proteins, histamine levels were measured in both supernatant and pellet fractions by fluorometric assay (36) using a model LS-3B Perkin-Elmer fluorescence spectrometer (Norwalk, CT). Histamine release was expressed as a percentage of the total cellular histamine content as calculated by the formula: (histamine in supernatant/histamine in supernatant and pellet) × 100. Spontaneous release of histamine was normally <2% of the total and was subtracted from the percent release to give the specific secretion shown in the text, tables, and figures. From dose-response curves of various secretagogues, the concentrations that specifically release 50% of the cellular histamine were calculated (EC50).

The secretagogues used in the studies included an extract of *N. brasiliensis* (8), the neuropeptide substance P (10–8 M), the ionophore A23187 (5 × 10–7 M), and a panel of defensins from humans, rabbits, and guinea pigs. Defensins were isolated from bone marrow for the rabbit (37) and guinea pig (38) peptides and from leukocytes obtained from the peritoneal exudate of patients with peritonitis for the human peptides (39, 40). The peptides were purified by reverse phase and size exclusion chromatography as previously described (37–40). The identity, purity, and quantity of each peptide were determined by amino acid analysis. Purified peptides were stored lyophilized at –20°C until use. The nomenclature and structure of the defensins used in this manuscript are summarized in Table I.

Isolation of mast cell membrane fraction

A mast cell membrane fraction was isolated as described previously (44). Briefly, purified PMC (30 × 106) were resuspended in homogenization buffer (HEPES buffer with 2 mM MgCl2, 1 mM ATP, 10 μg/ml PMSF, and a protease inhibitor mixture consisting of 5 μg/ml each of leupeptin, apronin, and tosyl-arginine methyl ester (TAKE)). Cells were ruptured by repeated passage (5–10 times) through a 12-μm clearance in a ball-bearing homogenizer (EMBL, Heidelberg, Germany), with resulting organelles removed by centrifugation at 400 × g for 5 min. The supernatant was then centrifuged at 100,000 × g for 1 h at 4°C. The membrane pellet was resuspended in a small volume of 10 mM triethanolamine/HCl buffer and was stored at –70°C until use.

GTPase assay

GTPase activity was assayed by the hydrolysis of phosphate, from [γ-32P]GTP as previously described (45). Briefly, a reaction mixture (100 μL) containing 50 mM triethanolamine/HCl (pH 7.5), 2 mM MgCl2, 1 mM DTT, 0.5 mM EGTA, 2 mg/ml BSA, 0.8 μM adenosine 5′-(β-imino)-triphosphate, 0.1 mM ATP, 0.4 μg/ml creatine kinase, 5 mM creatine phosphate, and 5 mM [γ-32P]GTP was prewarmed (5 min, 30°C). Reactions were started by addition of membrane fraction (1.0–2.0 μg of protein) to the prewarmed incubation mixture containing substance P or NP3a and allowed to proceed for 25 min at 30°C. The reaction was stopped by addition of 700 μL of 10 mM sodium phosphate (pH 2.0) containing 5% (w/v) activated charcoal. The tubes were chilled for 30 min on ice, then centrifuged at 13,000 × g for 10 min. A 500-μL aliquot was removed, and GTPase activity was evaluated by determination of Cherenkov radiation.

### Table I. The nomenclature and primary structures of defensins tested for mast cell degranulating activity

<table>
<thead>
<tr>
<th>Consensus</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>GACACRRRF-CPSERFSGYCRVNGARYVRCSSR</td>
</tr>
<tr>
<td>NP3a/CSI</td>
<td>GRCVCRQILCSYERRIDKIRGVPPFCCPR</td>
</tr>
<tr>
<td>NP3b/CSI</td>
<td>VVCACRRAL-CLPRERRAGFPCRGRHLPCC</td>
</tr>
<tr>
<td>Human</td>
<td>ACYRIPA-CIAQERRYGTCIQYQRPLWAFFC</td>
</tr>
<tr>
<td>HNP-1/HP1</td>
<td>VCSSRLVF-CRRLTVEQNCLIGVSFTYCCRV</td>
</tr>
<tr>
<td>HNP-4/HP4</td>
<td>RRCICRTTTRCIFYRPLGTCIFQONRVTYFCC</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>RPRFCFCLRLHCRC</td>
</tr>
<tr>
<td>GPNP-1/GPCSI</td>
<td>GPNP-1/GPCSI RRCICRTTTRCIFYRPLGTCIFQONRVTYFCC</td>
</tr>
<tr>
<td>GPCSI</td>
<td>GPNP-1/GPCSI RRCICRTTTRCIFYRPLGTCIFQONRVTYFCC</td>
</tr>
<tr>
<td>GPCIII</td>
<td>GPNP-1/GPCSI RRCICRTTTRCIFYRPLGTCIFQONRVTYFCC</td>
</tr>
</tbody>
</table>

The nomenclature and primary structures of defensins tested for mast cell degranulating activity.

### Notes

1. The guinea pig peptide GPCSIII is more closely related to the protegrin I. The guinea pig GPCSIII RRPRCFCRLHCRC family (41) of cysteine-rich cationic neutrophil peptides than to the classic defensins and exists as an antiparallel homodimer (38).

2. GTPase activity was evaluated by determination of Cherenkov radiation.
Statistical analyses

Data are given as the mean ± SEM. Differences among treatment groups were examined for significance by two-way ANOVA. Individual differences were assessed for statistical significance (p < 0.05) by paired or unpaired Student’s t test.

Results

Dose response and EC50 for defensins

The abilities of various concentrations of rabbit, guinea pig, and human defensins to induce histamine secretion from purified rat PMC were studied (Fig. 1). The rabbit defensins NP1, NP3a, and NP3b induced histamine secretion at low nanomolar concentrations and induced a maximum of 50–60% specific histamine release. The EC50 for these rabbit peptides varied from 70–620 nM (Table II). A similar pattern of histamine secretion occurred with guinea pig defensins, GPNP1 and GPCSIII (Fig. 1B and Table II).

The human defensins HNP1 and HNP4, were less potent in inducing histamine secretion from rat mast cells. At 2.5 μM the maximum histamine secretion was 40–55% with EC50 for HNP1 and for HNP4 of 2.9 and 2.2 μM, respectively (Fig. 1C and Table II).

Although the amounts of rat defensins available for study were limited, in a single experiment R2 (5 nM), R4 (0.5 nM), and R5 (0.5 nM) induced histamine secretion (15.2, 51.7, and 45.8%, respectively); R3 induced no histamine secretion.

The EC50 of the defensins studied generally correlated with the nominal net charge of the peptides at pH 7.4 as calculated from their known sequences (Table II). These values correlate with their relative charge as determined by acid-urea gel electrophoresis. Rabbit NP3a was the most potent inducer of specific histamine release (EC50 70 nM; Table II). Thus, for further studies of the mechanisms involved in histamine secretion with defensins we used NP3a as a representative peptide. For comparisons, the characteristics of histamine secretion induced by NP3a under different experimental conditions (time-course analysis and effects of pertussis toxin and neuraminidase) were compared with those of substance P, Ag, and the calcium ionophore A23187. Interestingly, NP3a did not induce histamine secretion from mucosal mast cells isolated (8) from rat small intestinal mucosa (results not shown).

Time course analysis of histamine secretion

Given that differences in the time course of histamine secretion occur with various secretagogues (polycations have a rapid time course, ≤10 s) and relate to the activation pathways involved, we studied the time course of histamine secretion induced by NP3a and other secretagogues. NP3a and the polycation substance P exhibited a rapid time course (<10 s required for maximum percent histamine release), whereas the time courses of Ag- and ionophore (A23187)-induced histamine secretion were delayed and reached a maximum after 20 or at least 60 s, respectively (Fig. 2).

Effects of pretreatment with pertussis toxin on histamine secretion

Histamine secretion induced by polycations such as 48/80 and substance P is highly sensitive to pertussis toxin, whereas Ag- and ionophore-induced histamine release is not (10, 13, 46–48).

Therefore, we investigated the effects of pertussis toxin on NP3a-induced histamine secretion (Fig. 3). Purified PMC were preincubated for 2 h with various concentrations (1–100 ng/ml) of pertussis toxin, washed, and then stimulated with various secretagogues for 10 min. Pertussis toxin (100 ng/ml) almost completely inhibited histamine secretion induced by substance P (94.5 ± 3.8% inhibition) and NP3a (96.3 ± 2.3% inhibition), whereas it was without effect on A23187-induced histamine secretion (data not shown) and only partially inhibited (48 ± 5.5%) Ag-induced histamine secretion. Inhibition was evident with as little as 10 ng/ml pertussis toxin during a 2-h pretreatment.

Effects of pretreatment with neuraminidase on histamine secretion

Histamine secretion induced by polycations, but not by A23187, is also highly sensitive to inhibition by the removal of sialic acid residues from the cell surface using neuraminidase (10). Thus, to characterize the sensitivity of NP3a-induced histamine secretion to
neuraminidase, we pretreated purified PMC with various concentrations (0.01–0.1 U/ml) of neuraminidase for 1 h, washed them, and then challenged them with various secretagogues (Fig. 4). NP3a- and substance P-induced histamine secretion was highly sensitive to the removal of sialic acid residues (80.0 ± 2.4% and 72.7 ± 8.0% inhibition, respectively). By contrast, histamine secretion induced by Ag stimulation was partially (33.8 ± 5.2%) inhibited by neuraminidase treatment, and there was no effect of neuraminidase on histamine secretion induced by A23187 (data not shown).

Enhancement of GTPase activity by NP3a

Because the activation of histamine secretion from mast cells by NP3a appeared to be similar to that of substance P and presumably other polycationic peptides, we studied whether NP3a could induce GTPase activity, a measure of activation of G proteins, in a membrane fraction of PMC. An isolated membrane fraction from purified PMC induced a significant (p < 0.05) GTPase response in the presence of 140 nM NP3a (7.7 ± 1.6 pmol/min/µg), consistent with or greater than the positive response to 10−5 M substance P (7.3 ± 1.7 pmol/min/mg; Fig. 5). This was reproducible in multiple studies with a single membrane preparation as well as with independent membrane preparations.

Discussion

Several cationic peptides, including venom peptides such as mast cell-degranulating peptide (MCDP), and neuroendocrine peptides such as substance P, vasoactive intestinal polypeptide, and somatostatin promote IgE-independent histamine release from mast cells. Rat intestinal mucosal mast cells often contact subepithelial substance P neurons (49), suggesting a physiological role for substance P-induced mast cell degranulation. This observation received strong support when Janiszewski et al. (9), showed that concentrations as low as 5 pM substance P primed mast cells for subsequent activation by substance P and other secretagogues. However, peptide-induced histamine release also may be an unwanted side effect in the development of pharmacologically useful peptide agents; for example, many luteinizing hormone-releasing hormone antagonists degranulate mast cells (50). Neutrophils elaborate low molecular mass (≤5 kDa) HRF whose identity is not known but which appears by biological and chromatographic criteria to differ from other biochemically characterized histamine-releasing polypeptides. Neutrophil-derived guinea pig defensins activate histamine release from mast cells (35). We therefore examined the histamine-releasing properties and mechanisms of action of several defensins from different species as candidate neutrophil-derived HRF.

The ability to degranulate mast cells is common to all defensins tested, with EC50 ranging from 70 nM to 2.9 µM. Defensins resemble wasp MCDP in terms of their cysteine-rich character and their potencies on rat PMC: 20 nM for MCDP (51, 52) and 70 nM for NP3a. On a molar basis the EC50 for the highly cationic defensin rabbit NP3a was 65.2 nM, similar to that of substance P (10−8 M), NP3a (140 nM), A23187 (5 × 10−6 M), or N. brasiliensis (five worm equivalents) Ag (mean ± SE; n = 3–6 experiments for each secretagogue). NP3a and substance P are significantly different from Ag and A23187 at 5 and 10 s (*, p ≤ 0.05).

### Table II. Maximum histamine secretion, EC50 and nominal net charge at pH 7.4 of defensins tested for mast cell degranulating activity

<table>
<thead>
<tr>
<th>Species</th>
<th>Peptides</th>
<th>Maximum Histamine Release (%)</th>
<th>Conc. (nM)</th>
<th>EC50 (nM)</th>
<th>Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>GPNPI</td>
<td>67.4 ± 1.7</td>
<td>830</td>
<td>100</td>
<td>+7</td>
</tr>
<tr>
<td></td>
<td>GPCSIII</td>
<td>68.8 ± 1.7</td>
<td>2500</td>
<td>300</td>
<td>+10</td>
</tr>
<tr>
<td>Human</td>
<td>HP-1</td>
<td>43.1 ± 3.2</td>
<td>2500</td>
<td>2870</td>
<td>+3</td>
</tr>
<tr>
<td></td>
<td>HP-4</td>
<td>55.4 ± 3.2</td>
<td>2500</td>
<td>2160</td>
<td>+4</td>
</tr>
<tr>
<td>Rabbit</td>
<td>NP3a</td>
<td>65.2 ± 1.8</td>
<td>420</td>
<td>70</td>
<td>+8</td>
</tr>
<tr>
<td></td>
<td>NP3b</td>
<td>55.0 ± 1.5</td>
<td>1260</td>
<td>240</td>
<td>+8</td>
</tr>
<tr>
<td></td>
<td>NP1</td>
<td>53.6 ± 3.1</td>
<td>1260</td>
<td>620</td>
<td>+9</td>
</tr>
</tbody>
</table>

FIGURE 2. Time-course analysis of specific histamine secretion induced by substance P (10−8 M), NP3a (140 nM), A23187 (5 × 10−6 M), or N. brasiliensis (five worm equivalents) Ag (mean ± SE; n = 3–6 experiments for each secretagogue). NP3a and substance P are significantly different from Ag and A23187 at 5 and 10 s (*, p ≤ 0.05).

FIGURE 3. Effects of pretreatment with pertussis toxin (2 h, 37°C) on the inhibition of specific histamine secretion stimulated by substance P (10−8 M), NP3a (140 nM), A23187 (5 × 10−6 M), or N. brasiliensis (five worm equivalents) Ag (mean ± SE; n = 3–6). NP3a and substance P are significantly different from Ag and A23187 at all doses of pertussis toxin (*, p ≤ 0.05).
noncysteinyl neuroendocrine peptides (8) such as somatostatin (EC$_{50}$ 2.0 µM), vasoactive intestinal polypeptide (EC$_{50}$ 6.0 µM), and substance P (EC$_{50}$ 40.0 µM) on rat PMC. The range of defensin EC$_{50}$ as mast cell secretagogues shows a strong correlation with the net charge of the peptide at pH 7.4; the more basic defensins are more potent mast cell-degranulating agents. The potency of mast cell degranulation also tends to follow the rank order of these peptides as inhibitors of the ACTH receptor (corticostatic activity), suggesting similarities in the underlying mechanisms of these two responses. HP-1 and HP-4, which have the weakest histamine-releasing activity are antimicrobial (54, 55), demonstrating that the structure-activity correlates that govern histamine secretion differ from those that determine microbicidal action.

In common with other cationic peptides, defensins degranulate mast cells through a pertussis toxin-inhibitable mechanism, implying an action on G proteins. The time course of histamine release by defensins is identical with that of substance P, and both peptides are inhibited by neuraminidase treatment of mast cells. Moreover, as with substance P, NP3a induces GTPase activity. Thus, the mechanism of defensin-induced mast cell degranulation is similar to that of other polycationic peptides. This is also consistent with the lack of effect of NP3a on histamine secretion from intestinal mucosal mast cells, as this mast cell subset is unresponsive to other polycations. This unresponsiveness may protect these mucosal mast cells from spurious activation by defensin-like cryptdins thought to be constitutively released from Paneth cells in the intestinal crypts (56). Alternately, cryptdins may activate mucosal mast cells, whereas neutrophil-derived defensins do not. Peptide-induced histamine release is thought to involve receptor-independent activation of G proteins (13). The details of how such peptides activate G proteins remain unclear, but involve direct interaction of the peptide with the G protein. Charge and membrane penetration are important; however, HP-1, which is membrane penetrating (57), only weakly activates mast cell histamine secretion. In studies on synthetic magainin analogues (amphipathic helical antimicrobial peptides from frog skin) Cross et al. (58) were able to uncouple strong histamine-releasing activity from peptide-induced membrane perturbations. The amino acid configuration may exert an important influence on peptide-induced mast cell degranulation, since L- to D-arginine substitutions in substance P analogues, which conserved overall charge, showed reduced mast cell activation (58).

There is growing evidence of important communication between mast cells and neutrophils in inflammatory reactions. Neutrophil recruitment to inflammatory sites is mast cell dependent (59), involves their production of TNF-α (60), and has important antimicrobial activities (61, 62). In addition, mast cell-derived tryptase has a pronounced effect on neutrophil recruitment (63, 64). In turn, neutrophil-derived factors have long been implicated in histamine release at sites of inflammation, and defensin-induced histamine release may contribute to this process. During infection, plasma defensin concentrations may greatly exceed the levels that induce degranulation of mast cells in vitro. In sepsis, cumulative concentrations in plasma of the human defensins HNP1–3 rise from 40 ng/ml in healthy individuals to as high as 170 ng/ml (65, 66). Highly elevated levels of HNP1–3 occur in sputum from cystic fibrosis patients (67) and in pleural effusions from patients with empyema (68). Solid tissue infiltrated with neutrophils may have total HNP1–3 levels up to 25 mmol/g wet weight or roughly 90 µg (39). Defensins are prominent within the extracellular spaces of experimentally induced syphilitic lesions in rabbits (69). The plasma level of rabbit NP3a, the most active MCDP, increases from 8 ng/ml in uninfected animals to 234 ng/ml during experimental peritonitis (70). The release of defensins may arise both from the lysis of expended neutrophils and from cytokine-stimulated secretion. IL-8 (64, 68) or PMA (68) stimulates the release of HNP1–3 from human neutrophils, and at least in pleural effusions the local levels of IL-8 exceed the concentrations required to promote the secretion of neutrophil defensins.

Since defensins are released from neutrophils in response to IL-8 and can be proinflammatory (71), these peptides may also be released by mast cell-derived TNF-α and tryptase and accumulate at sites of inflammation. Thus, it appears that this bidirectional communication between mast cells and neutrophils is important in acute inflammatory responses. It may involve important innate responses that help direct the kinds of immune and inflammatory responses that evolve following injury and Ag exposure and clearly requires further study. Regardless of the physiological or pathological outcomes of defensin-induced histamine release and inflammatory cascades, the design of novel antibiotics based on defensin structures will have to take account of their propensity to degranulate mast cells.
Acknowledgments

We thank Mrs. Fran Thompson for secretarial and editorial assistance. We also thank Drs. P. Lacy and F. L. Willis for their assistance with the GTPase assay.

References

   antigen-binding factor with in vivo activity analogous to IgE antibody. J. Exp.
   Med. 157:862.
   145:1851.
   activation for anaphylatoxins C3a stimulation of mast cells. J. Immunol.
   148:2459.
   Mast cell heterogeneity: effects of neuropeptides on histamine release.
   of substance P trigger electrical responses in mast cells without degranulation.
    activation: a receptor-independent mode of action for amphiphilic neuropeptides
    by basic secretagogues: evidence for direct activation of GTP-binding proteins.
    factors and cytokine-dependent activation of basophils and mast cells. Adv.
    Immunol. 50:237.
15. Sim, T. C., R. Alam, P. A. Forsythe, J. B. Welter, M. A. Lett-Brown, and
    J. A. Grant. 1992. Measurement of histamine-releasing factor activity in individ-
    ual nasal washings: relationship with atopy, basophil response, and membrane-
    fibrosis: identification of a histamine releasing factor in bronchoalveolar lavage.
17. Warner, J. A., M. M. Pienkowski, M. Plan, P. S. Norman, and
    L. Mousli. 1986. Identification of histamine releasing factor(s) in the late
    releasing factors in diisocyanate-induced occupational asthma. Am. J. Respir.
    Crit. Care Med. 150:988.
19. Nakamura, T., and M. Ui. 1985. Simultaneous inhibitions of inositol phospho-
    tides and cationic proteinase activities by islet-activating protein, pertussis toxin:
    a possible involvement of the toxin-sensitive G protein in rat mast cell degranula-


