Histamine release from gut mast cells from patients with inflammatory bowel diseases

H Nolte, N Spijelnaes, A Kruse, B Windelborg

Abstract
Inflammatory mediators from intestinal mast cells may serve as initiators of acute and delayed inflammation. Mast cell histamine release was measured in 19 patients with inflammatory bowel diseases using gut mast cells from enzymatically dispersed endoscopic forceps biopsy specimens of macroscopically inflamed and normal tissue. Mast cells and corresponding basophils were challenged with anti-IgE, anti-IgG, subclass anti-IgG4, and formyl-methionyl-leucyl-phenylalanine (FMLP) and results were compared with those from nine patient control subjects.

The mast cell count in patients with ulcerative colitis was increased compared with that in control subjects and patients with Crohn's disease, and the mast cell count obtained from inflamed tissue was greater than that of normal tissue. The study also shows the heterogeneity of the responsiveness of the histamine releasing cells to various secretagogues. Thus, mast cells released 0-4 (0-0-2-0) (median (range)) ng histamine per sample at anti-IgE challenge, and basophils were also anti-IgE responsive. In contrast, mast cells did not respond to FMLP but the corresponding basophils did. Gut mast cells released 0-3 (0-0-1-0) (median (range)) ng histamine per sample at anti-IgG4 challenge; however, the corresponding basophils did not respond to anti-IgG4. In addition, the anti-IgG4 mediated histamine release was primarily confined to patients with inflammatory bowel disease.

This study substantiates previous histopathological findings that mast cells may play a functional role in the inflammatory process of inflammatory bowel diseases and provides evidence for a possible role of subclass IgG4 as a reaginic antibody.

Materials and methods

PATIENTS AND PATIENT CONTROLS
Twenty-eight subjects were included in the study after informed consent had been given. Eighteen patients with active inflammatory bowel disease were classified into Crohn's disease and ulcerative colitis according to clinical features, endoscopic findings, and histopathological examination. The nine control subjects were patients being examined for adenoma. In the patient control subjects, 10 endoscopic forceps biopsy specimens (Olympus biopsy forceps FB-13 u) of mean (SD) 10-3 (0-2) mg were obtained. In patients with inflammatory bowel disease five biopsy specimens of 10-8 (0-6) mg were obtained from macroscopically normal and inflamed tissue. Some 5 ml EDTA-anticoagulated blood were collected by venepuncture in all subjects.

REAGENTS
Anti-IgE (461.620 U/ml) was obtained from Behringwerke (West Germany). Monoclonal antibody against IgG4 subclass was obtained from Unipath (Bedford, United Kingdom) and polyclonal rabbit antihuman IgG from Dakopatts (Copenhagen, Denmark). N-formyl-methionyl-leucyl-phenylalanine (FMLP) was obtained from Sigma. Collagenase (0-15 U/mg) from Boehringer-Mannheim. The histamine releasing agents were diluted in Pipes-AMC buffer.16 Glass microfibre prepared microtitre plates were provided by Lundbeck Diagnostics (Copenhagen, Denmark).

MAST CELL DISPERSION
The biopsy specimens were dispersed mechanically and enzymatically, as described elsewhere. Briefly, the specimens were immediately transferred to 5 ml Pipes-AMC at 4°C. Within one hour they were dispersed during incubation for 90 minutes at 37°C in a buffered salt solution containing collagenase (0-015 U/ml), penicillin (75 U/ml), streptomycin (75 μg/ml), and supplemented with 20% fetal calf serum. After incubation, the cells were washed three times and pooled in 1-0 ml of Pipes-AMC. The mast cell number was assessed by light microscopy using Kimura's metachromatic stain, and mast cell numbers were determined in a Bürker-Türk haematocytometer. The mast cells looked granulated and structurally intact. Cell viability, as assessed by trypan blue exclusion, was greater than 95% in dispersed cells. The mast cells were resuspended to a final concentration of 7×10⁶ mast cells/ml (SD 1-8×10⁶/ml). Then mast...
cells from inflamed and normal tissue were incubated separately.

**Basophil and Mast Cell Histamine Release**

Histamine release from mast cells and basophils was measured as previously described.\(^9\) Briefly, 50 μl 'washed' EDTA-anticoagulated blood was placed in a glassfibre prepared microtitre plate. In the wells of another microtitre plate, 30 μl dispersed mast cell samples were placed.

The samples were incubated for 60 minutes at 37°C at the following final dilutions: FMLP at 1×10\(^{-4}\) M, 10\(^{-4}\) M, and 10\(^{-5}\) M, anti-IgE at 1, 100, and 1000 U/ml, and polyclonal anti-IgG and monoclonal anti-IgG\(_4\) at 10\(^{-4}\), 10\(^{-5}\), and 10\(^{-6}\) M. After incubation histamine was measured by the fluorometric O-pthalaldehyde method.\(^8\) The histamine release was calculated on the basis of a histamine standard curve included in all experiments. The histamine released from unstimulated samples (spontaneous release) was subtracted from the histamine released from stimulated samples to give the net stimulated histamine release.\(^9\) A histamine release of >0.1 ng histamine per sample was considered significant.\(^*\)

**Statistics**

The Mann-Whitney test was used when comparing the mast cell counts. The Wilcoxon signed rank test was used when comparing the values for each patient. The tests of significance, median, range, mean, and standard deviation reported are calculated from the mean (SD) for triplicate determinations.

**Results**

**Patients and Tissue Samples**

Mucosal specimens from nine patients with ulcerative colitis (median age 31 years; range 17–53 years) and 10 patients with Crohn’s disease (median age 36 years; range 26–41 years) were obtained by endoscopic biopsy (Table I). Two patients with ulcerative colitis were treated with 5-aminosalicylic acid and one patient with Crohn’s disease was given oral steroids. These patients, however, did not release histamine from either mast cells or basophils.

**Average Mast Cell Yields**

No significant difference in dispersed mast cell count compared with normal controls was found in normal tissue (macroscopically and histologically verified) from patients with Crohn’s disease (Table II). In contrast, an increased mast cell count was found in normal tissue from patients with ulcerative colitis compared with patient control subjects (p<0.05). In addition, the mast cell count obtained from inflamed tissue was greater than that from normal tissue (p<0.01).

**Mast Cell Histamine Release**

**Anti-IgE.** Fifteen of 28 subjects released histamine to anti-IgE challenge and mast cells released histamine from both inflamed and normal tissue (Table III). Histamine release tended to be increased in inflamed tissue from patients with Crohn’s disease, but this was not significant (p>0.05, Table IV).

**Subclass IgG.** Polyclonal anti-IgG did not elicit histamine release in any subjects (results not shown). In contrast, monoclonal anti-IgG\(_4\) induced significant (>0.1 ng histamine/sample) histamine release in 10 patients with inflammatory bowel disease and one patient control subject (Table III). The mast cell histamine release from inflamed tissue compared with normal tissue was significantly increased (Table IV, p<0.05).

**FMLP.** Incubation of mast cells with FMLP failed to induce a significant increase in histamine release above spontaneous values.

**Comparison Between Mast Cells from Non-Inflamed and Inflamed Tissue**

Table III shows that more patients responded with mast cell histamine release to anti-IgE and anti-IgG\(_4\) challenge from inflamed tissue than

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**TABLE I** Patient characteristics

<table>
<thead>
<tr>
<th>Patient group</th>
<th>M:F</th>
<th>Age (yr) median (range)</th>
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</thead>
<tbody>
<tr>
<td>Patient control subjects (n=9)</td>
<td>5:4</td>
<td>47 (35-69)</td>
</tr>
<tr>
<td>Ulcerative colitis (n=9)</td>
<td>5:4</td>
<td>31 (17-53)</td>
</tr>
<tr>
<td>Crohn’s disease (n=10)</td>
<td>7:3</td>
<td>36 (26-41)</td>
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</tbody>
</table>

**TABLE II** Mast cell counts per mg tissue (median (range))

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Normal tissue</th>
<th>Inflamed tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient control subjects</td>
<td>1250 (0-1750)</td>
<td>2000 (1500-2500)</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>1500 (0-2250)</td>
<td>1750 (1500-2250)</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>1250 (0-2500)</td>
<td>2000 (1500-3500)</td>
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</tbody>
</table>

**TABLE III** Number of subjects releasing >0.1 ng histamine/sample in response to the various secretagogues

<table>
<thead>
<tr>
<th>Mast cells</th>
<th>Basophils</th>
<th>Tissue: non-inflamed</th>
<th>Tissue: inflamed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient control subjects (n=9)</td>
<td>Anti-IgE</td>
<td>8</td>
<td>7</td>
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<tr>
<td></td>
<td>Anti-IgG(_4)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>FMLP</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Ulcerative colitis (n=9)</td>
<td>Anti-IgE</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Anti-IgG(_4)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>FMLP</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Crohn’s disease (n=10)</td>
<td>Anti-IgE</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Anti-IgG(_4)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>FMLP</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

FMLP=formyl-methionyl-leucyl-phenylalanine.
Mast cell histamine release

From normal tissue. Hence, 10 patients with inflammatory bowel disease released histamine from inflamed tissue mast cells at anti-IgG4 challenge but the corresponding normal tissue mast cells showed histamine release in four patients only.

With regard to anti-IgE, there was a discrepancy in responsiveness between normal tissue mast cells and inflamed tissue mast cells in two patients only. One patient who did not respond to anti-IgE challenge showed mast cell histamine release at anti-IgG4 challenge solely.

**Basophil Histamine Release**

**Anti-IgE.** Twenty four of 28 subjects showed basophil histamine release to anti-IgE challenge. The anti-IgE mediated basophil histamine release compared with mast cell histamine release was increased in patient control subjects (p<0.05). No such difference was found between mast cells and basophils in patients with inflammatory bowel disease (Tables IV and V).

**Anti-IgG.** None of the subjects released histamine from basophils at challenge with subclass anti-IgG4 or polyclonal IgG.

**FMLP.** FMLP caused histamine release in basophils. Compared with anti-IgE mediated basophil histamine release, the number of subjects responding to FMLP was smaller and the amount of histamine (ng/sample) was also decreased (p<0.05).

**Comparison Between Corresponding Basophils and Mast Cells.**

The response to mast cell anti-IgE challenge in patients with Crohn's disease was noticeably reduced compared with results obtained from their corresponding basophils. Although mast cells did not respond to FMLP, their corresponding basophils released histamine on FMLP challenge. In contrast, anti-IgG4 induced mast cell histamine release and no basophil histamine release.

**Discussion**

This study shows that gut mast cells may release histamine in response to various secretagogues and may thus play a functional role in the inflammatory process of inflammatory bowel disease. To assess immunologically stimulated histamine release, unsensitised dispersed mast cells were challenged with anti-IgE. The results indicate the presence of functional IgE in the intestinal mast cells allowing immunological cell activation.

It is known that human basophils and mast cells may also bind to IgG antibodies other than IgE. Some authors suggest that allergen specific IgG4 may react as allergen neutralising antibodies. There is, however, controversy over whether IgG4 antibodies adhere to relevant membrane receptors on mast cells. It is surprising that subclass anti-IgG4 was able to trigger histamine release from gut mast cells. The results suggest, however, the presence of functional Fc receptors and the presence of not only IgE but also IgG4 in gut mast cells. The anti-IgG4 mediated histamine release, however, was predominantly confined to patients with inflammatory bowel disease. This suggests that mast cells in these patients possess higher amounts of IgG4 than IgE compared with normal subjects. A noticeable local IgG response, with an increase in the proportion of IgG immunocytes, has previously been found in inflammatory bowel disease.7 Attention has been focused on a local IgG response as a "second line" of mucosal defence, however, and a local pathogenetic effect of subclass IgG4 antibody has not been considered. Thus, the histamine releasing effect of anti-IgG4 may reflect a pathogenetic role of IgG4 antibodies in inflammatory bowel disease. To elucidate this finding further, passive sensitisation with subclass IgG4 antibodies should be performed.13 The corresponding basophils were not responsive to anti-IgG4, confirming previous findings.13

FMLP is a potent histamine secretagogue in human basophils and these results show that in mast cell dispersates no significant histamine release was induced. This indicates that dispersates were not contaminated with basophils. Furthermore, our results show the heterogeneity between human basophils and mast cells with regard to the difference in response to various inducers of histamine release as previously found.13-17

In conclusion, the microtitre assay of histamine is a convenient method of studying the functional aspects of intestinal mast cells in patients and the method may also prove useful in investigating the effect of various drugs on dispersed gut mast cells from patients with inflammatory bowel disease as well as elucidating part of the immunological mechanisms in these diseases.

We thank Mrs E M Raaby for her skilful technical assistance and the nurses in the endoscopic laboratory for their help in collecting the tissue specimens.

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**Table V**

<table>
<thead>
<tr>
<th>Patients groups</th>
<th>Basophil histamine release (ng/sample) (mean (range))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-IgE basophil</td>
</tr>
<tr>
<td>Patient control subjects</td>
<td>1-4 (0-2-0)</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>0-7 (0-1-8)</td>
</tr>
<tr>
<td>Crohn's disease</td>
<td>0-8 (0-2-3-2)</td>
</tr>
</tbody>
</table>


