PLASMA HISTAMINE AND TUMOUR NECROSIS FACTOR-ALPHA LEVELS IN CROHN’S DISEASE AND ULCERATIVE COLITIS AT VARIOUS STAGES OF DISEASE

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Mast cells secrete numerous mediators and this study investigated plasma levels of histamine, and tumor necrosis factor alpha (TNF-α) in chronic inflammatory bowel disease (IBD). Plasma levels of histamine were determined in 68 patients with Crohn’s disease (CD), 22 with ulcerative colitis (UC) and 13 controls. TNF-α levels were assessed in 29 CD patients, 11 UC patients, and in 11 controls. Plasma histamine levels in the control group were 0.25 ng (0.14 – 0.33) and showed no difference to CD (0.19 ng, 0.09 – 0.35) or UC (0.23 ng, 0.11 – 0.60). Significantly lower histamine levels were only found in CD patients on 5-aminosalicylic acid treatment (P ≤ 0.04). Plasma TNF-α levels in the control group were significantly lower 0.44 ml/m² (0 – 1.15) than in CD patients (4.62 ml/m², 1.82 – 9.22, P = 0.005) or UC (3.14 ml/m², 0.08 – 11.34, P = 0.01). In CD disease activity, fistula, and extraintestinal manifestations (EM) were associated with significantly higher plasma TNF-α values, but not the type of treatment. We concluded that in contrast to TNF-α, histamine levels were normal in CD and UC. There is no correlation with histamine and thus the proportion of TNF-α secreted from mast cells in the plasma in patients with IBD is less important.

Key words: plasma histamine, tumor necrosis factor-α, inflammatory bowel disease, Crohn’s disease, ulcerative colitis

INTRODUCTION

Today, idiopathic chronic inflammatory bowel diseases (IBD), Crohn’s disease (CD), and ulcerative colitis (UC) are thought to be caused by defects in the interplay between genetic predisposition, exogenous factors (intestinal flora, nutrition, physical activity and smoking), and a dysregulated immune response with chronic activation of immune and inflammatory cells (1-3). CD involves discontinuous transmural inflammation that can affect the entire gastrointestinal tract. The ileocecal region is affected most frequently. By definition UC starts in the rectum and spreads continuously through the mucosa and submucosa in a proximal direction, at worst developing into pancolitis with or without backwash ileitis. Both diseases can be associated with diverse extraintestinal manifestations (EM), which in CD often also involve formation of fistulas (1, 2). IBD is usually diagnosed serologically, clinically, endoscopically, pathohistologically, and radiologically with respect to the presence of certain pathophysiological inflammatory signs. Its extent of disease is often quantified by endoscopy and the clinical disease activity documented by using scientific scores.

According to more recent investigations, local and systemic inflammatory activity is essentially caused by various interleukins (IL-1, IL-6, IL-12, IL-17, etc.), leptin and tumor necrosis factor alpha (TNF-α). TNF-α can be synthesized and secreted by many kinds of cells after cell activation via NFκB (e.g., monocytes, neutrophilic granulocytes, and T lymphocytes) but mast cells and basophilic granulocytes also are also capable of producing TNF-α (1, 2, 4-6). Mast cells actually contain TNF-α in preformed vesicles and can secrete TNF-α together with histamine, one of its main mediators, within minutes (5, 6). Interestingly, TNF-α - similarly to histamine - can alter vascular and intestinal permeability and TNF-α can also stimulate the proliferation of smooth intestinal muscle cells and fibroblasts, which can cause typical IBD lesions such as bowel wall thickening, stricture, and stenosis (2, 6-11). The local accumulation of mast cells has been reported in particular for strictures and inflammatory lesions in IBD and thus a pathogenetic link could exist between TNF-α and histamine in CD and/or UC (6-8). Indeed, although up to now no causative or curative medical treatment exists for IBD, inhibitory properties to suppress mast cell activity have been reported for conventional therapies such as aminosalicylates, corticosteroids, dietary treatment, and immunosuppressants and for more rarely used drugs such as antihistamines and mast cell stabilizers (5-8).

Considerably higher numbers of mast cells have been found in affected areas in both CD and UC patients than in nonaffected areas in the same patient or in healthy bowels (5-10). In addition, mast cells in CD patients contain much more TNF-α than do those in UC patients or controls; mast cells secrete more histamine in IBD; and the histamine content is significantly increased in jejunal perfusion fluid in CD patients (6-10). Since histamine has a very short half-life of 1 – 2 min in blood, rapid and precise processing is needed to determine plasma levels of this compound. Several studies have postulated an important role for mast cells and histamine in IBD (6-10); however, except for one treatment study no systematic investigations using precise methods have been conducted to determine the magnitude of plasma histamine levels in IBD (8, 13). Therefore, the goal of the present study was to investigate whether 1) there is a correlate for functional mast cell activity at the systemic plasma level and 2) whether by comparison...
of histamine with the known proinflammatory cytokine TNF-α, any indications for a central pathogenetic relevance of mast cells for IBD can be found in the plasma compartment. To this end, by using a cross-sectional study design, nonselected IBD patients were investigated as regards inflammatory activity, anatomical extent of inflammation, presence of EM, and medications.

MATERIAL AND METHODS

Patients

This study was approved by the local ethics committee (No. 925) and all patients gave their informed consent for the mediator diagnostic studies. We included a total of 91 out- and in-patients and IBD inpatients who had already received a diagnosis of CD (n = 69) or UC (n = 22) according to serological, clinical, endoscopic, radiological, and histological examinations before entering the study (Table 1). The study patients were referred to the University Erlangen, Department of Medicine 1, for follow-up examinations, during acute episodes, to exclude neoplasias, owing to new complaints, or to adjust their medication. Patients in the IBD group were classified according to their current medication (no treatment, 5-aminosalicylic acid, steroidal, or immune suppressants), disease activity (CDAI, CAI), and for the presence of EM or fistula.

The control group comprised 13 healthy individuals and clinic staff who were not on any medication and who were not suffering from IBD, allergy, or an infection (Table 1).

Exclusion criteria for the study were drugs that could induce a nonspecific release of histamine such as antibiotics, NSARs, opioids, muscle relaxants, or blood products. Furthermore, pregnant women (false-negative plasma histamine concentrations (10)), patients on anticoagulants, and those being treated with anti-TNF-α antagonists were excluded from the present study.

Determining mediators of histamine and tumor necrosis factor-α in plasma

Testing for plasma mediators in the blood was performed in fasting patients between 7 and 9 a.m. All subjects had eaten normally the previous day (no diet) but had fasted from 6 p.m. onwards. Fasting conditions for drawing blood were particularly important because certain foods (cheese, wine, sauerkraut, etc.) contain histamine, and exogenous histamine can produce false-positive results for histamine levels in plasma (6-8, 12).

The EDTA blood (S-Monovette®, Sarstedt, Numbrecht, Germany) was cooled on ice (4°C) immediately after being drawn from the vein in order to avoid any possible histamine degradation (12, 13). Then, the blood samples were centrifuged immediately for 10 min at 4000 U/min and cooled (4°C) so that the plasma could be separated from the erythrocytes. The plasma samples were frozen at −20°C in separate batches for histamine and TNF-α testing until the ELISA assay could be performed to quantitatively determine levels of those compounds (12-14). To avoid false-positive results for histamine, we took care that no hemolytic, icteric, or lipemic samples were frozen: when certain concentrations are exceeded (hemoglobin 5 mg/mL, bilirubin 1 mg/mL, triglycerides 30 mg/mL), cross-reactions with methylhistamine and acetylhistamine can occur during the ELISA assay (13, 14).

Plasma histamine and TNF-α levels were each determined photometrically by carrying out special ELISA tests according to the manufacturer’s instructions and controlled according to standard samples and curves (12-14). The test sensitivity for histamine is 0.05 ng/ml and that for TNF 2.3 pg/ml (13, 14). Coefficients of intra- and interassay variation for more than 20 plasma samples using ELISA to detect histamine were 3.2 and 4.1%, respectively. For TNF-α this intra- and interassay variation was 7.1 and 13.6%, respectively.

Statistical tests

For statistical testing, the software Graph Pad Instat 3 and graphics program Graph Pad Prism Version 4.00, April 3, 2003, were used. The t-test was employed to analyze normal distribution for age of controls and patients in two independent samples. To test for significance in comparing the median values in the various patient groups nonparametric U-tests for independent samples (Mann-Whitney test) were applied. A test result with an error probability of P < 0.05 was considered statistically significant. Pearson correlation analyses for independent samples were used to calculate the correlation between histamine and TNF-α in the plasma and for activity indices with histamine and TNF-α. Here, the correlation coefficient r and coefficient of determination r² were calculated.

RESULTS

The age of patients with CD (inactive, active, according to the Crohn’s Disease Activity Index < 150 points or > 150 points), UC (inactive, active, according to Rachmilewitz Colitis Activity Index < 4 points or > 4 points), and controls showed a normal distribution and at P > 0.05 no statistically significant differences were found between the individual groups (Table 1).

However, for both histamine and TNF-α, the mediator plasma levels mostly did not show a normal distribution in the disease groups as compared to controls. Therefore, we worked with mean values and 25 – 75% percentiles.

Plasma histamine in Crohn’s disease and ulcerative colitis

An overview of the median plasma concentrations of histamine (ng histamine/ml × m² body surface area) can be found in Table 2. By standardizing the plasma levels of histamine to ng histamine/ml × m² body surface area, a normal range of 0.14 – 0.33 for histamine was calculated in the control group with a variance of approx. 47.7%.

Compared to the median of the controls (0.25) no significant changes in plasma levels of histamine were found for CD (0.19) and UC (0.23) even after differentiating between inactive and active disease state. However, the plasma histamine levels varied to a considerably greater degree in all disease groups than in healthy controls (variance 63.6 – 164.7%, Table 2).

By using our strict, standardized methods to calculate the normal range of laboratory values for plasma histamine from the mean value in the control group and two standard deviations (mean + 2 S.D., 0.24 + 2 × 0.11 = 0.46), we found a relatively narrow normal range at P ≤ 0.46.

The distribution for individual histamine concentrations in the normal controls showed that none of the 13 healthy individuals (0%) had values above the normal range of 0.46. For CD, 11 of 68 patients (16.1%) showed values above the calculated normal range of 0.46 (inactive CD, 6/31 patients 19.3%; active CD, 5/37 13.5%). Among the UC patients, 6 of 22 subjects (27.2%) showed values higher than 0.46 (inactive UC 6/12 patients 50%; active UC 0/10 0.0%).

Effect of inflammatory bowel disease medications on plasma levels of histamine

Analysis of plasma histamine levels in IBD patients with respect to medication showed that patients not on treatment
tended to have higher plasma levels of histamine (Table 3) than those being treated with 5-aminosalicylic acid, steroids, or immune suppressant drugs. Significantly lower levels of plasma histamine as compared to the controls (P = 0.04) and to untreated patients with CD were only observed, however, in patients with CD who were taking 5-aminosalicylic acid.

The UC group tended to have higher plasma levels of histamine; however, these values were not statistically significantly different from those of controls or CD patients (Table 3).

Effect of disease localization on plasma levels of histamine

Disease localization of IBD did not have a statistically significant effect on the concentration of histamine in plasma (Table 4). Indeed, for both CD and UC patients in whom the affected region of the bowel was precisely indicated, the median plasma histamine levels for proctitis, colitis, ileitis, ileocolitis, or left-sided colitis and pancolitis were in the normal range (under 0.46).

Effect of extraintestinal manifestations and fistula on plasma levels of histamine

Similar, nonsignificant results were found for the plasma concentrations of histamine in patients with CD and EM (0.20; 0.09 – 0.43, n = 27) or fistula (0.18; 0.08 – 0.38, n = 14). This lack of significance also held true after differentiating between active and inactive disease (P > 0.05 in all subgroups).

In the four patients with UC and EM, the plasma levels of histamine tended to be higher at 0.39 (0.18 – 0.70), but this was not significantly different from the control group values.

### Table 1. Patients characteristics.

<table>
<thead>
<tr>
<th>Disease Group</th>
<th>n = pts</th>
<th>Sex f/m</th>
<th>Age (median)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>13</td>
<td>7/6</td>
<td>38.0</td>
<td>19 – 68</td>
</tr>
<tr>
<td>CD</td>
<td>68</td>
<td>39/29</td>
<td>43.8</td>
<td>23 – 78</td>
</tr>
<tr>
<td>CD inactive</td>
<td>31</td>
<td>16/15</td>
<td>42.7</td>
<td>26 – 68</td>
</tr>
<tr>
<td>CD active</td>
<td>37</td>
<td>21/6</td>
<td>44.8</td>
<td>23 – 78</td>
</tr>
<tr>
<td>UC</td>
<td>22</td>
<td>11/11</td>
<td>38.3</td>
<td>19 – 60</td>
</tr>
<tr>
<td>UC inactive</td>
<td>12</td>
<td>7/5</td>
<td>37.6</td>
<td>19 – 51</td>
</tr>
<tr>
<td>UC active</td>
<td>10</td>
<td>5/5</td>
<td>38.8</td>
<td>22 – 60</td>
</tr>
</tbody>
</table>

n - number of patients (pts.), f - female, m - male, CG - control group, CD - Crohn’s disease, inactive CDAI < 150 points, active CDAI >150 points, UC - ulcerative colitis, inactive CAI < 4 points, active CAI > 4 points.

### Table 2. Plasma histamine levels (ng/ml × m² body surface area) in inflammatory bowel disease and controls.

<table>
<thead>
<tr>
<th>Histamine</th>
<th>n = pts</th>
<th>Median 25% percentile</th>
<th>75% percentile</th>
<th>mean ± S.D.</th>
<th>coefficient variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>13</td>
<td>0.25</td>
<td>0.14</td>
<td>0.33</td>
<td>0.24 ± 0.11</td>
</tr>
<tr>
<td>CD</td>
<td>68</td>
<td>0.19</td>
<td>0.09</td>
<td>0.35</td>
<td>0.31 ± 0.5</td>
</tr>
<tr>
<td>CD inactive</td>
<td>31</td>
<td>0.15</td>
<td>0.08</td>
<td>0.35</td>
<td>0.30 ± 0.5</td>
</tr>
<tr>
<td>CD active</td>
<td>37</td>
<td>0.19</td>
<td>0.09</td>
<td>0.38</td>
<td>0.32 ± 0.4</td>
</tr>
<tr>
<td>UC</td>
<td>22</td>
<td>0.23</td>
<td>0.11</td>
<td>0.60</td>
<td>0.34 ± 0.3</td>
</tr>
<tr>
<td>UC inactive</td>
<td>12</td>
<td>0.43</td>
<td>0.22</td>
<td>0.82</td>
<td>0.50 ± 0.4</td>
</tr>
<tr>
<td>UC active</td>
<td>10</td>
<td>0.17</td>
<td>0.05</td>
<td>0.24</td>
<td>0.15 ± 0.1</td>
</tr>
</tbody>
</table>

### Table 3. Plasma histamine levels (ng/ml × m² body surface area) in inflammatory bowel disease in relation to medication.

<table>
<thead>
<tr>
<th>Histamine Group</th>
<th>No medication</th>
<th>5-ASA</th>
<th>steroids</th>
<th>immuno-suppressants</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>n = 10</td>
<td>n = 15</td>
<td>n = 31</td>
<td>n = 10</td>
</tr>
<tr>
<td></td>
<td>(0.12 – 0.65)</td>
<td>(0.121,2)</td>
<td>(0.12 – 0.40)</td>
<td>(0.07 – 0.37)</td>
</tr>
<tr>
<td>UC</td>
<td>n = 2</td>
<td>n = 9</td>
<td>n = 8</td>
<td>n = 3</td>
</tr>
<tr>
<td></td>
<td>(0.62 – 0.83)</td>
<td>(0.23)</td>
<td>(0.08 – 0.52)</td>
<td>(0.22 – 0.83)</td>
</tr>
</tbody>
</table>

1 P = 0.04 versus controls and 2 P = 0.03 versus CD without medication; 5-ASA 5-aminosalicylic acid.
Table 4. Plasma histamine levels (ng/ml × m² body surface area) in inflammatory bowel disease in relation to disease location and extent.

<table>
<thead>
<tr>
<th>Histamine Group</th>
<th>Proctitis</th>
<th>Colitis</th>
<th>Ileitis</th>
<th>Ileocolitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD n = 4</td>
<td>0.19 (0.17 – 0.73)</td>
<td>0.20 (0.13 – 0.28)</td>
<td>0.14 (0.07 – 0.23)</td>
<td>0.16 (0.08 – 0.38)</td>
</tr>
<tr>
<td>UC n = 4</td>
<td>0.40 (0.13 – 0.72)</td>
<td>0.21 (0.08 – 0.26)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 5. Plasma TNF-α levels (ng/ml × m² body surface area) in inflammatory bowel disease and controls.

<table>
<thead>
<tr>
<th>TNF-α Group</th>
<th>n = pts</th>
<th>median</th>
<th>25% percentile</th>
<th>75% percentile</th>
<th>mean±S.D.</th>
<th>coefficient variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>11</td>
<td>0.44</td>
<td>0.00</td>
<td>1.15</td>
<td>1.14 ± 1.9</td>
<td>166.1</td>
</tr>
<tr>
<td>CD inactive1</td>
<td>29</td>
<td>4.62</td>
<td>1.82</td>
<td>9.22</td>
<td>5.91 ± 4.6</td>
<td>78.7</td>
</tr>
<tr>
<td>CD active1</td>
<td>13</td>
<td>4.09</td>
<td>1.20</td>
<td>10.13</td>
<td>5.99 ± 5.2</td>
<td>87.5</td>
</tr>
<tr>
<td>UC</td>
<td>11</td>
<td>3.44</td>
<td>0.08</td>
<td>14.64</td>
<td>6.19 ± 5.6</td>
<td>127.4</td>
</tr>
<tr>
<td>UC inactive</td>
<td>4</td>
<td>3.03</td>
<td>0.06</td>
<td>7.91</td>
<td>3.71 ± 3.1</td>
<td>100.6</td>
</tr>
<tr>
<td>UC active</td>
<td>7</td>
<td>4.12</td>
<td>0.60</td>
<td>14.46</td>
<td>4.88 ± 4.9</td>
<td>113.3</td>
</tr>
</tbody>
</table>

Significance: 1 P = 0.005, 2 P = 0.01, 3 P = 0.008 versus control group.

Table 6. Plasma TNF-α levels (ng/ml × m² body surface area) in inflammatory bowel disease in relation to medication.

<table>
<thead>
<tr>
<th>TNF-α Group</th>
<th>No medication</th>
<th>5-ASA</th>
<th>Steroids</th>
<th>immuno-suppressants</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD n = 7</td>
<td>4.091 (1.10 – 7.50)</td>
<td>n = 10</td>
<td>8.222 (2.28 – 13.54)</td>
<td>n = 17</td>
</tr>
<tr>
<td>UC n = 3</td>
<td>1.67 (0.08 – 3.21)</td>
<td>n = 6</td>
<td>4.01 (0.96 – 14.64)</td>
<td>n = 3</td>
</tr>
</tbody>
</table>

1 P = 0.05 versus controls and 2 P < 0.007 versus controls; 5-ASA 5-aminosalicylic acid. There was no significant difference between the groups with and without medication either in CD or UC.

**Plasma levels of tumor necrosis factor-α in Crohn’s disease and ulcerative colitis**

Table 5 presents an overview of the median plasma levels of TNF-α (pg TNF-α/ml × m² body surface area). Standardizing the TNF-α levels to pg/ml × m² body surface area, the control group showed a normal range of TNF-α from 0.0 – 1.15, with a wide variance in these values of approx. 166.1%. In comparison to the controls (0.44), TNF-α concentrations were significantly higher at, respectively, 10-fold and eightfold in the CD (4.62) and UC (4.42) patients, who also showed a lower variance than did the control group (78.7%). After differentiating between inactive and active disease states, the difference between the CD patients and controls was still statistically significant. In the UC patients, no significant changes could be observed owing to the low number of samples; however, patients with active disease tended to have higher levels of TNF-α, similarly to the CD patients.

By using our strict, standardized methods to calculate the normal range of laboratory values for TNF-α from the mean value in the control group and two standard deviations (mean ± 2 SD, 1.14 + 2 × 1.9 = 4.94), we found a normal range at P ≤ 4.94.

The distribution of the individual plasma levels of TNF-α in healthy controls shows that only in one of the 11 healthy individuals (9.0%) were values above the normal range of 4.94. For 14 of 29 CD patients, corresponding to 48%, values were above the calculated normal range of 4.94 for plasma TNF-α (inactive CD 6/13 patients, 46.1%; active CD 8/16, 50.0%). For 5 of 11 UC patients (45.4%), values were above the normal range of 4.94 (inactive UC 2/4 patients, 50.0%; active UC 3/7 patients, 42.8%).

**Effect of inflammatory bowel disease medication on plasma levels of tumor necrosis factor-α**

With respect to medication, plasma TNF-α levels in CD patients were lower in patients not on treatment (Table 6) than in those being treated with 5-aminosalicylic acid, steroids, or immunosuppressants. Indeed, plasma TNF-α levels were significantly higher in all treated groups than in the controls (P <
Comparison of the nontreated CD group and the treated subgroups did not show any statistically significant differences, however. A similar result was observed for plasma TNF-α levels in the UC group, which, however, did not reach statistical significance owing to the low number of patients. Nontreated patients also presented lower TNF concentrations than those on treatment with 5-aminosalicylic acid or steroids.

**Effect of disease localization on plasma tumor necrosis factor-α levels**

The localization of chronic inflammation in the bowel did not have any significant effect on plasma TNF-α levels in CD patients (Table 7) as the concentrations of TNF-α did not increase statistically significantly with an increase in affected areas in the bowel in these patients. For the individual affected areas in CD patients, consistently higher values than in controls were observed again, however (P < 0.03).

For UC a statistically significant increase in plasma TNF-α levels was found for both subtotal and total colitis (P < 0.03).

**Plasma TNF-α levels (ng/ml × m² body surface area) in inflammatory bowel disease in relation to extraintestinal manifestations and fistula.**

<table>
<thead>
<tr>
<th>TNF-α Group</th>
<th>Without EM</th>
<th>With EM</th>
<th>Fistula</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1</td>
<td>n = 17</td>
<td>n = 11</td>
<td>n = 7</td>
</tr>
<tr>
<td></td>
<td>4.46</td>
<td>6.26</td>
<td>6.26</td>
</tr>
<tr>
<td></td>
<td>(0.86 – 9.22)</td>
<td>(2.30 – 13.06)</td>
<td>(1.27 – 13.00)</td>
</tr>
<tr>
<td>UC</td>
<td>n = 91</td>
<td>n = 3</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>4.08</td>
<td>4.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.89 – 12.6)</td>
<td>(0.42 – 8.99)</td>
<td></td>
</tr>
</tbody>
</table>

1 P < 0.03 compared with control group.

No correlation was found between plasma histamine levels and disease activity in CD patients. For UC, a low-grade negative correlation with significance was established (P = 0.02, Table 9).

**Effect of extraintestinal manifestations and fistulas on plasma levels of tumor necrosis factor-α**

Plasma TNF-α levels were again significantly higher in CD patients both with (P = 0.005) and without (P = 0.01) EM and in patients with fistulas than in the controls (P < 0.01, Table 8). Between the patient groups presenting with and without EM, however, no statistically significant differences were observed in CD.

In the three patients with UC and EM, a mean plasma TNF-α level of 4.78 was found, which, however, did not differ from that of controls in a statistically significant manner.

**Correlation between plasma levels of histamine and tumor necrosis factor-α with disease activity in Crohn’s disease and ulcerative colitis patients**

No correlation was found between plasma histamine levels and disease activity in CD patients. For UC, a low-grade negative correlation with significance was established (P = 0.02, Table 9).
For TNF-α, a significant correlation with CDAI could not be found for CD patients either. For UC, a low-grade correlation with CAI was observed, which, however, did not reach significance (Table 9).

Furthermore, neither of the mediators investigated, plasma levels of histamine and TNF-α, correlated significantly with each other for CD (r² = 0.01, P > 0.05) or for UC (r² = 0.14, P > 0.05).

DISCUSSION

The role of mast cells in IBD has still not been clearly elucidated (5, 11). Since about 1975 reports have been appearing about increased degranulation of increasing numbers of mast cells in IBD tissue. Recently even mast cells loaded with TNF-α, IL-16-positive mast cells, substance P-positive mast cells, and potent protease-secreting mast cells have been discovered in bowel tissue from IBD patients by immunohistochemistry (8-11, 16-19). Although these findings speak for a local contribution of mast cells to the inflammatory process with the secretion of histamine, tryptase, cytokines, and other mediators, no systematic studies have been conducted to measure the plasma levels of histamine in the blood of patients with CD or UC.

By using a precise technique with a rapid, cooled processing of samples so as to avoid histamine degradation to determine plasma levels of histamine and standardizing these values with respect to body surface area (ng histamine/ml × m² body surface area), we found a relatively narrow normal range for plasma histamine at below 0.46 in a healthy control group. The median histamine levels in the patient groups of CD and UC did not differ from those of the controls. Thus, for our study patients with chronic IBD the plasma levels of histamine were not increased at a systemic level, although contrasting findings of increased mast cell counts, degranulation, or high concentrations of mediators locally in the bowel have been reported elsewhere (8-10, 16-19). Considering individual plasma levels of histamine, too, only about 16% of CD patients and about 27% of UC patients presented with systematically elevated plasma histamine levels that were outside the standard normal range (Fig. 1). Thus, systemic effects of histamine are only to be expected in a small proportion of patients with IBD. In order to determine whether the few individuals who present with systemically elevated plasma levels of histamine that are well outside of the normal range represent people with allergies, atopic disease, or with a reduced capacity to enzymatically degrade histamine and are among those patients with CD or UC, further investigations need to be conducted (6-8, 10, 17-22). Indeed, higher plasma levels of histamine tended to be found among the UC patients. This is in line with literature reports of a higher frequency of atopic diseases, denser eosinophilic infiltration in the bowel tissue, Th2 immune regulation, and higher serum levels of IgE in UC than in CD patients (7, 8, 23).

Numerous subgroup analyses could not establish any difference in plasma histamine levels with respect to disease localization, extent of inflammation, presence of EM or fistula; with respect to medications; however, plasma histamine levels tended to be higher in untreated IBD patients than in those being treated. Indeed, 5-aminosalicylic acid significantly lowered plasma histamine levels (Table 3) whereas steroids and immunosuppressants did not help to lower these levels significantly in either CD or UC patients. The inhibitory effect of 5-aminosalicylic acid on mast cells and basophils was previously experimentally demonstrated (24). It remains unknown, however, why 5-aminosalicylic acid treatment only reaches significance in CD and not UC patients. Possibly the aforementioned, tendentially higher plasma histamine levels in UC and the predominating immunological mechanisms of increased Th2 immune regulation and stronger mast cell activation in UC patients may play a decisive role (7, 9, 10, 17, 25).

It was previously demonstrated that, in CD, disease activity correlates significantly with urine excretion of methylhistamine in contrast to the findings here dealing with plasma histamine levels (6); therefore, this may indicate, at least for CD, that the histamine released locally in the bowel is rapidly methylated in bowel tissue, endothelium and in the liver and thus that high systemic plasma histamine levels cannot be expected (6, 12, 21, 22). Therefore, the present results with plasma histamine can only be considered in terms of their potential systemic effect. Indeed, the local rate of intestinal histamine release, the tissue metabolic rate, and the proinflammatory effect of histamine as a local tissue hormone cannot be estimated by plasma histamine determinations, owing to the different compartments of bowel and blood plasma (6-8, 19,20).

![Fig. 1](image_url). Frequency of IBD patients with significantly increased plasma mediator levels above the normal range.
In direct comparison with histamine, this study demonstrated a much greater systemic significance for TNF-α than for histamine in IBD. In contrast to the histamine findings, a significantly increased, systemically effective plasma TNF-α concentration was found for both CD and UC (Table 5). Previous studies also reported an increased production of TNF-α in bowel tissue and subsequent detection of increased TNF-α levels in stool and also systemically in serum. The greater systemic role of TNF-α is also highlighted by the association of IBD with certain polymorphisms of the TNF gene and the known correlation between soluble TNF receptors in urine and disease activity (1, 11, 26-30). In agreement with numerous other studies, which have reported a better therapeutic response to anti-TNF antibodies in CD than in UC, we found higher TNF-α levels and considerably more pronounced, statistically significant differences in the CD group than in the UC group. This applies both to the CD group as a whole as well as to active and inactive disease states of CD 1, 29-32), for patients with and without EM, and fistulas.

In spite of the significantly increased median plasma TNF-α concentrations in the IBD cohort, the individual TNF-α levels were only far outside the normal range for healthy individuals in about 48% of CD and 40% of UC patients. In direct comparison with histamine, the clearly more important role of TNF-α emerges here, too (Fig. 1). These data also show that inflammation in IBD does not develop uniformly via the proinflammatory cytokine TNF in all patients, but rather that additional heterogeneous mechanisms exist that induce and promote the inflammation (e.g., IL-6, IL-17, IL-18, IL-23, etc.) (1, 2, 5, 11, 33).

No significant differences in TNF-α plasma concentrations were observed either with respect to the different disease localizations or the extent of inflammation in CD patients. However, for TNF-α a significant contrast to histamine was found concerning medication. Indeed, IBD patients currently not on treatment had the lowest TNF-α levels and the treated patient groups presented with significantly higher levels. Neither on patients on 5-aminosalicylic acid nor for those on steroids or immunosuppressants could a reduction in plasma TNF-α levels to the normal range be found (Table 6). However, this was a cross-sectional study of IBD patients in various stages of disease, and thus we cannot clarify whether patients with active disease had possibly had even higher TNF-α levels before treatment was initiated than those measured while they were receiving medication. On the other hand, the TNF-α results with respect to medication could also be interpreted to mean that IBD patients who were not receiving any medication had gone into remission and could stop taking the medication because the pronounced inflammatory activity and hence TNF-α production had dropped so decidedly. Similar to the results of other investigations, we did not find a correlation between plasma TNF-α levels and disease activity either for CD or UC (30, 33, 34).

This may in part be due to the limitations of the present study; heterogeneous patients in various stages of disease and receiving different medications were analyzed using a cross-sectional study design. In addition, systemically effective mediator levels and the disease activity indices used measure different aspects of chronic bowel inflammation, different compartments were compared with each other, and the significance of the various types of immune cells secreting TNF-α may be different for CD and UC (fibroblasts, macrophages, epithelial cells, paneth cells, mast cells, neutrophils, and eosinophilic granulocytes). Furthermore, numerous other immune mechanisms may need to be taken into consideration (1, 2, 5, 7, 26, 30, 32-35).

In our study, we did not find a correlation between plasma levels of histamine and TNF-α; nor were quantitatively similarly pronounced changes in IBD patients observed. Bearing in mind the different compartments involved, bowel-blood plasma, these investigations at the blood plasma level could not demonstrate a predominant role of mast cells or of histamine for IBD (1, 2, 5, 32). The previous reports of heterogenous treatment effects of antihistamines, mast cell stabilizers, or anti-allergic treatments could perhaps be explained by the low rate of IBD patients presenting with plasma histamine levels outside of the normal range (16 – 27%) or an associated atopic or allergic disease (5-8, 13, 23). However, histamine and TNF-α seem to be secreted independently of each other in IBD, which means that varying types of active immune cells are involved in the expression of inflammation, with a clearer, but not exclusive predominance of TNF-α (1, 11, 34-38). Since only about 50% of all patients with chronic bowel inflammation demonstrate systemically increased plasma levels of TNF-α, future long-term clinical studies should determine in which patients expressed TNF-α mechanisms predominately contribute to disease manifestations, which measures actually can normalize TNF-α levels, and most of all, what other cytokine mechanisms exist in those individuals who do not show increased TNF-α production either locally or systemically (11, 30-32, 37, 38).

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REFERENCES


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