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Circulating levels of chemerin are elevated in inflammatory bowel disease

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Abstract

Chemerin is an adipose tissue-secreted protein that stimulates chemotaxis of cells of the innate immune system. Inflammatory bowel disease (IBD) is linked to an impaired immune response and, therefore, we hypothesized that systemic chemerin may be altered in IBD-patients. Serum was collected from patients with Crohn’s disease (CD, 230 patients), ulcerative colitis (UC, 80 patients) and healthy controls (HC, 80 probands). Chemerin and adiponectin, which has already been measured in the serum of similar cohorts by others, were determined by ELISA. Systemic chemerin concentrations were significantly elevated in serum of CD and UC patients compared to HC, and were also found to be higher in the serum of males with CD compared to males with UC. Adiponectin levels were lower in CD compared to UC and HC with similar circulating concentrations. In serum of male but not female patients chemerin levels were higher in UC patients with active disease whereas adiponectin was reduced. In CD elevated chemerin was associated with remission in males only. Treatment with corticosteroids was linked to elevated adiponectin in male CD patients and higher chemerin in female UC patients. Unlike adiponectin that is elevated in female serum in all cohorts analysed, chemerin was only higher in the serum of female UC patients. These data indicate that the levels of circulating chemerin are elevated in patients suffering from UC and CD whereas adiponectin is reduced in the latter. Relations of the systemic
concentrations of these adipokines to disease activity and treatment are disease- and gender-specific.
Introduction

Inflammatory bowel disease (IBD) with the two major forms Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory disorders of the gastrointestinal tract [1, 2]. In patients with Crohn’s disease mesenteric adipose tissue hypertrophy is a characteristic feature of this disease [3] whereas submucosal fat deposition (fat halo sign) is known in CD and UC [4]. Adiponectin is an adipose tissue-derived protein with protective effects in diseases associated with low grades of chronic inflammation like type 2 diabetes or atherosclerosis [5, 6]. Adiponectin deficiency protects mice from chemically-induced colitis in one study but leads to more severe colitis in a second investigation [7, 8]. Systemic adiponectin was measured in the serum of IBD patients and was found either increased in UC patients only, or decreased in CD and UC patients compared to the respective controls [9, 10].

Chemerin is also released by adipose tissue [11, 12] but has not been analysed in IBD so far. Chemerin is secreted as an inactive precursor. Proteolysis by serine proteases converts it to an agonist of the orphan G-protein coupled receptor chemokine-like receptor 1 (CMKLR1, ChemR23) [13-15]. ChemR23 is highly abundant on plasmacytoid dendritic cells (DC), macrophages, and CD56lowCD16+ natural killer cells [11, 13, 16, 17], and binding of chemerin stimulates chemotaxis [13]. Although chemerin was initially described as a proinflammatory adipokine, chemerin-derived peptides exert antiinflammatory activities indicating that chemerin may play a role in the initiation and termination of inflammation [18, 19]. Exposure of neutrophils to chemerin reduces transepithelial migration, whereas activation of the epithelial ChemR23 stimulates apical clearance of neutrophils indicating a function of chemerin in the resolution of acute mucosal inflammation [20].

Unlike adiponectin that is significantly higher in female serum [5, 21], chemerin demonstrates no gender dimorphism in a cohort of type 2 diabetic patients (own unpublished observation),
and in 55 healthy volunteers [22]. Systemic chemerin levels were found similarly abundant in the serum of type 2 diabetic patients and normal-glucose tolerant controls [12], and this is in opposite to adiponectin whose circulating concentrations are reduced in obesity and insulin resistance [3, 21].

The purpose of the present study was to determine circulating adiponectin and chemerin in inflammatory bowel disease and to analyse whether these adipokines correlate with disease severity.
Materials and Methods

Material
Adiponectin and chemerin ELISAs were from R&D Systems (Wiesbaden-Nordenstadt, Germany).

ELISA
ELISAs were performed as recommended by the distributor. Serum was diluted 1 to 5,000-fold for adiponectin and 1 to 250-fold for chemerin determination.

Probands and Patients
All serum samples were obtained from Caucasians. The study protocol was approved by the local ethics committee and the investigation conforms to the principles outlined in the Declaration of Helsinki (1997). Each proband gave written informed consent to participate in the study. Details of the study groups are given in table 1. Healthy controls were recruited from the general population.
Diagnosis was based on endoscopic, histological and radiological findings, as reported by the diagnosing physician, in conjunction with symptoms suggestive of IBD as recently described [23]. Patients were classified as having UC if they presented with (i) typical clinical symptoms and (ii) continuous mucosal inflammation affecting the rectum and some or the entire colon in continuity seen by radiology or endoscopy with histopathological findings compatible with UC. CD was defined if the patient met two or more of the following features: (i) typical clinical symptoms, (ii) macroscopic segmental inflammation, the appearance of ‘cobblestone’ in affected segments, ulcerations, stenosis and penetrating lesions, (iii) stenosis of the bowel, fistulae or intra abdominal abscesses documented by radiology and (iv)
histopathological findings with transmural inflammation or epithelia granulomas with giant cells.

The Vienna classification [24] was applied to describe clinical patient subgroups in CD with A1: age at diagnosis < 40 years, A2: age at diagnosis > 40 years, L1: disease limited to the terminal ileum with or without spill into coecum, L2: disease location at any position between coecum and rectum without involvement of the small bowel or upper gastrointestinal tract, L3: involvement of the terminal ileum and the colon and L4: any disease location proximal to the terminal ileum, B1: non-stricturing, non-penetrating, B2: stricturing, B3: penetrating.

Remission was defined as an Crohn’s disease activity index < 150 in CD, and a clinical activity index ≤ 4 for UC as described [25].

Statistics

Data are presented as median and range of the values (SPSS 15.0 for Windows). Statistical differences were calculated by two-tailed Mann-Whitney U Test, and a value of p < 0.05 was considered as statistically significant. The Pearson's correlation was calculated using the SPSS 15.0 software.
Results

Association of chemerin and adiponectin with the body mass index (BMI), age, and gender

Chemerin and adiponectin were determined by ELISA in the serum of patients with Crohn’s disease (CD), ulcerative colitis (UC) and healthy controls (HC) (Table 1). Serum adiponectin negatively correlated with the BMI ($r = -0.23, p > 0.001$) of the probands whereas chemerin did not correlate with the BMI when all probands were included in the analysis or when the cohorts were analysed separately. Systemic adiponectin is elevated in females [5], and this was also identified in the whole study group and when CD, UC and HC were analysed individually. Chemerin levels were similar in the sera of males and females of the HC and the CD group but were significantly elevated in female UC patients ($p < 0.001$) (Fig. 1a). A positive correlation of chemerin and adiponectin was only identified in female UC patients ($r = 0.45, p < 0.001$, Fig. 1b).

Chemerin and adiponectin serum concentrations in IBD

Chemerin levels were significantly higher in the serum of CD and UC patients compared to HC ($p < 0.001$) when the whole study group was analysed, and when calculations were performed for female and male probands separately (Fig. 2a, c). Chemerin concentrations were also significantly elevated in CD compared to UC and gender-specific analysis revealed that chemerin was significantly increased in CD in comparison with UC in male patients only (Fig. 2a). Adiponectin levels were significantly reduced in male and female CD patients compared to UC and HC with similar circulating concentrations (Fig. 2b, d). Increased levels of adiponectin were found in serum of UC patients in relation to HC when the whole study
group was analysed and are explained by the higher number of females in the UC group. The detailed results are listed in table 1.

Correlation of chemerin and adiponectin to disease activity and Vienna Classification

Chemerin was found reduced in the serum of male UC patients and induced in the serum of male CD patients with non-active disease but was not related to disease activity in females (Fig. 3a, c and data not shown). Adiponectin was reduced in the serum of male UC patients with active disease (Fig. 2b, d). Neither adiponectin nor chemerin were related to the relevant classifying aspects of CD defined by the Vienna classification (data not shown). A positive correlation of chemerin with the duration of the disease was identified in male CD patients ($r = 0.363, p < 0.001$).

Treatment and systemic adipokines

Systemic adiponectin was elevated in male UC ($p = 0.008$) and CD patients ($p < 0.001$) with corticosteroid treatment. In contrast, chemerin was significantly elevated in female UC patients treated with corticosteroids ($p = 0.017$). Antibiotic drugs were related to higher adiponectin in the serum of male CD patients ($p = 0.027$) and azathioprine-treatment to elevated adiponectin in female CD patients ($p = 0.004$).
Our data demonstrate that systemic chemerin is elevated in IBD and reaches higher levels in male CD patients whereas adiponectin is specifically reduced in the serum of male and female CD patients. Unlike adiponectin that is significantly higher in serum of females, chemerin demonstrates no gender dimorphism [5, 21]. In accordance with these studies chemerin was similar in CD patients and HC of both sexes but was elevated in the serum of female UC patients compared to male UC patients indicating disease- and gender-related differences in systemic chemerin levels. Furthermore, chemerin positively correlated with adiponectin only in the serum of female UC patients.

Karmiris et al. [10] described significantly elevated adiponectin levels in UC whereas Valentini et al. [9] identified reduced adiponectin in the serum of CD and UC patients. These different observations may in part be explained by elevated adiponectin in females [5] and an unbalanced distribution of the sexes in the study cohorts. To circumvent this problem in our study, we performed also comparisons for both genders separately.

Adiponectin and chemerin are released from adipocytes, but whereas adiponectin is preferentially produced by these cells, chemerin is also synthesized by other cells like fetal intestinal epithelial cells or hepatocytes [26, 27]. Comparison of different fat depots revealed that visceral fat produces higher amounts of adiponectin compared to subcutaneous adipose tissue [28]. Mesenteric hypertrophic adipose tissue characteristic for CD patients even secretes more adiponectin than normal mesenteric fat of the identical patient [29]. In addition, adiponectin release from creeping fat of CD patients is elevated compared to mesenteric adipose tissue of patients with colon cancer or diverticulitis [30]. These findings might indicate that circulating adiponectin is higher in CD patients but systemic adiponectin was even found reduced indicating that locally released adiponectin of mesenteric adipose tissue is not relevant in determining systemic levels.
Chemerin is a chemoattractant for cells of the innate immune system but systemic levels have not been analysed in IBD so far. This chemokine is involved both in the initiation and resolution of inflammation indicating that altered levels may contribute to a disturbed immune response [18, 19]. In CD an inappropriate immune response in the mucosa and in macrophages derived from peripheral monocytes has been described [2, 31]. The immune response in local skin inflammation is also reduced indicating a generalized impaired innate immune response in these patients [2]. Systemic chemerin was similarly elevated in female and male IBD patients compared to HC and was further induced in male CD in relation to male UC patients. Circulating chemerin was not associated with disease activity in females but was slightly higher in male UC patients with active disease and more significantly reduced in male CD patients on remission. Chemerin is released as an inactive precursor protein and is activated by serine proteinases of the coagulation and fibrinolytic cascades [15, 32] that are more active in IBD [33]. Therefore, it may well be that higher systemic chemerin in IBD is associated with elevated levels of active chemerin. Epithelial cells respond to chemerin and upregulate CD55, that mediates clearance of neutrophils [20]. CD55-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis, and therefore, induction of CD55 by chemerin may protect epithelial cells from exaggerated inflammation [34]. However, more detailed studies that especially reveal the function of chemerin in the intestine are necessary to fully understand the role of elevated chemerin in IBD.

Systemic adiponectin serum levels were elevated in male UC and CD patients with steroid treatment, and azathioprine treatment was associated with elevated adiponectin in female CD patients. Corticoid ingestion in healthy men for one week increased systemic adiponectin [35] and immunosuppressant therapy is also linked to elevated adiponectin concentrations [36] indicating that the beneficial effects of these drugs in IBD may also include a rise in circulating adiponectin. Antibiotics were related to higher adiponectin in the serum of male
CD patients in accordance with a recent study in mice where antibiotics increased circulating adiponectin [37].

Chemerin, however, was significantly elevated only in the serum of female UC patients treated with corticosteroids. Dexamethasone in combination with 1,25 dihydroxivitamin D3 induces chemerin in osteoclast-supporting stromal cells [38] but the effects of steroids alone on chemerin expression have not been analysed in detail so far.

In summary, the current study revealed elevated chemerin levels in the serum of IBD patients with the highest levels in the serum of male CD patients. In contrast adiponectin was selectively reduced in the serum of male and female CD patients. Relation of the systemic concentrations of these adipokines to disease activity and treatment regimens were disease- and gender-specific indicating that gender may be highly relevant in IBD pathophysiology and medication.
Acknowledgement

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References


Figure legends

Fig. 1. Systemic chemerin in male and female IBD patients and healthy controls. (a) Chemerin in the serum of female (f) and male patients with CD, UC and healthy controls (HC). (b) Correlation of chemerin with adiponectin in female UC patients.

Fig. 2. Gender- and disease-specific protein levels of chemerin (a) Chemerin in the serum of male patients suffering from CD or UC, and in healthy controls (HC). (b) Adiponectin in the serum of male patients suffering from CD or UC and in healthy controls (HC). (c) Chemerin in the serum of female patients suffering from CD or UC and in healthy controls (HC). (d) Adiponectin in the serum of female patients suffering from CD or UC and in healthy controls (HC).

Fig. 3. Relation of adiponectin and chemerin to disease activity (a) Chemerin in male patients with active and non-active UC. (b) Adiponectin in male patients with active and non-active UC. (c) Chemerin in male patients with active and non-active CD. (d) Adiponectin in male patients with active and non-active CD.
Table 1: Characteristics of patients with Crohn’s disease, Ulcerative colitis and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Crohn’s disease</th>
<th>Ulcerative colitis</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Number</td>
<td>230</td>
<td>80</td>
<td>80</td>
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<tr>
<td>Females %</td>
<td>53</td>
<td>43</td>
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<tr>
<td>Age (years)</td>
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<td>42 (21–77)</td>
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<td>BMI (kg/m²)</td>
<td>22.9 (15.2–57.8)</td>
<td>22.9 (15.2–39.1)</td>
<td>23.1 (17.2–36.3)</td>
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<tr>
<td>Active/Inactive</td>
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<td>31/49</td>
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<tr>
<td>Disease duration</td>
<td>12 (4–40)</td>
<td>11 (3–28)</td>
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<tr>
<td></td>
<td>(years)</td>
<td></td>
<td></td>
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<tr>
<td>Vienna</td>
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<td>Classification</td>
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<td>A1 / A2</td>
<td>202 / 28</td>
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<td>Adipokines</td>
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<tr>
<td>Adiponectin</td>
<td>3.2 (1.0–12.6)</td>
<td>4.6 (1.5–13.7)</td>
<td>3.4 (1.0–14.0)</td>
<td>&lt;0.001¹, &lt;0.001²</td>
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<td>(µg/ml)</td>
<td>140 (63–388)</td>
<td>124 (59.6–230)</td>
<td>88.7 (42–214)</td>
<td></td>
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<tr>
<td>---------</td>
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<td></td>
</tr>
<tr>
<td>Chemerin (ng/ml)</td>
<td>0.024&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.003&lt;sup&gt;1&lt;/sup&gt;, 0.001&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&lt;0.001&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
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</table>

1: Significance CD – UC  
2: Significance CD – HC  
3: Significance UC – HC
Figure 3

A

B

C

D

Chemerin (ng/ml)

Adiponectin (µg/ml)

p=0.041

p=0.035

p=0.02

Active

Non-Active

Active

Non-Active

Active

Non-Active