Increased Circulating Levels of Interleukin-1 Receptor Antagonist in Patients with Inflammatory Bowel Disease

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Summary: Plasma levels of interleukin-1 receptor antagonist (IL-1ra), a potent inhibitor of IL-1, were measured in patients with inflammatory bowel disease. Plasma IL-1ra levels in patients with active ulcerative colitis and Crohn’s disease were higher than in normal controls. No significant difference was noted in plasma IL-1ra concentrations between active ulcerative colitis and Crohn’s disease patients. The levels in patients with inactive disease were lower than in active patients, but were higher than in normal controls. Plasma IL-1ra levels correlated significantly with clinical disease activity and laboratory parameters such as C-reactive protein or leukocyte count. In conclusion, circulating IL-1ra in patients with inflammatory bowel disease may be a useful marker of disease activity.

Key words interleukin-1, interleukin-1 receptor antagonist, ulcerative colitis, Crohn’s disease

INTRODUCTION

The causes of inflammatory bowel disease (IBD)—that is ulcerative colitis (UC) and Crohn’s disease (CD)—remain unknown, though an immune mechanism is involved [1,2]. It is now well established that several cytokines with potent proinflammatory activities, including interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α, are increased in IBD, indicating that these cytokines mediate many immunopathologic events [3-5]. In particular, the role of IL-1 has been extensively investigated, and it has been suggested that it is essential for the development of disease [6-10].

IL-1 is a 17 Kd polypeptide that exerts a number of immunostimulatory and inflammatory effects [5,11]. In addition to activating T lymphocytes, this powerful molecule has a wide range of systemic effects that include induction of fever, anorexia, neutrophilia, hypoferremia, and hypozincemia. It also stimulates the release of pituitary hormones, the synthesis of acute phase proteins, and the production of inflammatory eicosanoids or cytokines [5,11].

Recently, a specific inhibitor of IL-1, termed IL-1 receptor antagonist (IL-1ra), has been identified, and its cDNA has been cloned [12-14]. IL-1ra is closely related to IL-1α and IL-1β and competitively blocks the binding of IL-1 to its receptor [13,14]. IL-1ra has no agonist activity [15] and efficiently blocks IL-1 effects both in vitro and in vivo [13,14].

The recent demonstration of IL-1ra mRNA and its protein product in IBD colonic mucosa [8,11,16] has stimulated interest both in its participation in the pathophysiology of IBD and perhaps in its potential use as a new therapeutic agent. More recently, a genetic association was also reported between UC and IL-1ra [17]. However, little is known regarding the appearance of IL-1ra in the circulation. For these reasons, we measured the plasma levels of IL-1ra using a specific and sensitive enzyme-linked immunosorbent assay (ELISA).
TABLE 1.
Clinical characteristics of patients with inflammatory bowel disease

<table>
<thead>
<tr>
<th></th>
<th>UC</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>55</td>
<td>41</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>24/31</td>
<td>15/26</td>
</tr>
<tr>
<td>Age (yr)*</td>
<td>35.1 (15-67)</td>
<td>31.3 (16-66)</td>
</tr>
<tr>
<td>Duration of disease (yr)*</td>
<td>4.8 (1-21)</td>
<td>4.5 (1-16)</td>
</tr>
<tr>
<td>Disease activity (active/inactive)*</td>
<td>33/22</td>
<td>25/16</td>
</tr>
<tr>
<td>Site of disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total colon</td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>Left side of colon</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Distal colon</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Prednisolone plus sulfasalazine</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>None</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

*Mean (range). **Clinical disease activity of ulcerative colitis (UC) according to the criteria of Truelove and Witts and of Crohn’s disease (CD) according to the IOIBD score.

PATIENTS AND METHODS

Patients and controls

Blood samples were collected and analyzed for plasma IL-1ra levels in 55 patients with UC and 41 with CD. The diagnoses were based on characteristic clinical, endoscopic, radiologic and histologic criteria. Disease activity of UC was analyzed according to the criteria of Truelove and Witts and was classified as inactive, mild, moderate, or severe [18]. Disease activity of CD was assessed by the score of the International Organization for the Study of Inflammatory Bowel Disease (IOIBD) in which a score of 0-1 was defined as inactive disease and a score of 2 or greater as active disease [19]. The characteristics of the patient population are given in Table 1.

Twenty-four volunteers with an age range and sex distribution similar to the IBD patients served as normal controls. The disease control group comprised nine patients with infectious colitis and two patients with ischemic colitis. This project was performed according to the Helsinki Declaration and informed consent was obtained from every participant.

ELISA for IL-1ra

IL-1ra was measured by a sandwich ELISA developed by Amersham International plc (Biotrak, UK) using a mouse anti-human IL-1ra monoclonal antibody for capture and a goat anti-human IL-1ra polyclonal antibody for detection. The lower limit of detection was 22.0 pg/mL for plasma samples. This IL-1ra ELISA is highly specific and is not affected by IL-1α, IL-1β or any other major cytokine.

Determination of other cytokines and laboratory parameters

Plasma IL-1α or IL-1β levels were not measured in this study since it has been made clear that these levels were below the detection limit in a large number of IBD patients [13]. Plasma soluble IL-2 receptor (sIL-2R) was quantitated by ELISA (Boehringer Mannheim Biochemica, Germany) with a lower detection limit of 6.0 pmol/L. C-reactive protein (CRP) was measured by laser nephelometry (Hoechst Japan, Tokyo, Japan). The total leukocyte count, hemoglobin concentration, platelet count and erythrocyte sedimentation rate (ESR) were determined by standard procedures.

Statistics

Continuous variables among the various groups were compared by using analysis of variance and Fisher's least significant difference test after checking that the distribution was normal; if this was not the case, the Kruskal-Wallis test was used. For paired data the Wilcoxon signed rank test was used. Correlations between plasma IL-1ra levels and the score of IOIBD or laboratory measurements were tested using the Spearman rank correlation coefficient. Differences were considered significant at P<0.05.
RESULTS

Plasma levels of IL-1ra

The plasma IL-1ra levels in patients with active UC and CD were significantly higher than those of healthy controls (P<0.001 for both). There was no significant difference in IL-1ra levels between active UC and CD. The levels in patients with inactive UC and CD were significantly lower than in patients with active disease (P<0.01 and P<0.05, respectively), but were higher than in normal controls (P<0.005 for both). The levels in disease controls were significantly higher than in healthy controls (P<0.001) (Fig. 1). The analysis of paired samples showed a significant reduction of the plasma IL-1ra level in every patient with either form of IBD after clinical improvement (P<0.05 for both) (Fig. 2).

Relationship of IL-1ra or sIL-2R plasma levels to clinical and laboratory parameters

There was a statistical association between the IL-1ra level and clinical parameters in patients with

Fig. 1. Plasma levels of IL-1ra in patients with ulcerative colitis (UC) and Crohn’s disease (CD), normal controls, and disease controls. Each point represents the value in a single individual. n: number of patients.

Fig. 2. Serial levels of plasma IL-1ra in patients with ulcerative colitis (A) and Crohn’s disease (B). Paired plasma samples were obtained during active and inactive stages of disease and assayed for IL-1ra by ELISA.

Fig. 3. Relationship between plasma IL-1ra levels and clinical disease activity, as assessed by the criteria of Truelove and Witts in ulcerative colitis (A) and by the score of the International Organization for the Study of Inflammatory Bowel Disease (IOIBD) in Crohn’s disease (B).
TABLE 2. Correlation of IL-1ra or sIL-2R plasma levels with laboratory parameters

<table>
<thead>
<tr>
<th></th>
<th>IL-1ra</th>
<th>sIL-2R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UC</td>
<td>CD</td>
</tr>
<tr>
<td>Leukocyte counts</td>
<td>0.531b</td>
<td>0.667a</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.168</td>
<td>0.060</td>
</tr>
<tr>
<td>Platelet counts</td>
<td>0.045a</td>
<td>0.612a</td>
</tr>
<tr>
<td>1-h ESR</td>
<td>0.154</td>
<td>0.633</td>
</tr>
<tr>
<td>CRP</td>
<td>0.731a</td>
<td>0.576a</td>
</tr>
<tr>
<td>sIL-2R</td>
<td>0.552a</td>
<td>0.279</td>
</tr>
</tbody>
</table>

The numbers represent the correlation coefficients (r value) between laboratory parameters indicated in the left column and plasma IL-1ra or sIL-2R concentrations.

a P<0.001, b P<0.005, c P<0.01, d P<0.05. UC: ulcerative colitis; CD: Crohn's disease; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; sIL-2R: soluble interleukin-2 receptor

both UC and CD (UC, P<0.005, Kruskal-Wallis test; CD, r=0.646, P<0.001, Spearman rank correlation test) (Fig. 3). A similar association was observed between the sIL-2R level and clinical parameters (UC, P<0.0001; CD, r=0.618, P<0.001) (data not shown). Table 2 summarizes the correlation coefficients and significance values between plasma IL-1ra levels and each indicated laboratory parameter. There was a significant correlation between the IL-1ra level and laboratory parameters, such as CRP or leukocyte count, in patients with these diseases. In addition, a comparison between these parameters and plasma sIL-2R levels in the same patients was also shown.

DISCUSSION

This study shows that patients with active IBD had increased circulating levels of IL-1ra compared with healthy controls. The levels were lower in patients with inactive IBD than in patients with active disease, but remained higher than in healthy controls, suggesting either the presence of underlying immunologic activation or slow clearance of this circulating protein. However, the latter is probably unlikely since recent longitudinal studies in patients undergoing surgery [20] or during experimental endotoxemia [21] have demonstrated the rapid restoration of IL-1ra to its baseline level after the initial elevation of circulating IL-1ra.

We next compared the circulating IL-1ra level to clinical and laboratory parameters of disease activity. We found a statistical association between IL-1ra levels and clinical disease activity or laboratory parameters such as CRP or leukocyte count in both diseases. We also confirmed the significance of the correlation of circulating sIL-2R levels with clinical disease activity and some of the laboratory parameters, as shown in previous studies [22,23]. These results may be of interest since there is great need for a relevant marker of IBD activity.

Although the mechanism for regulating the IL-1ra release in IBD is unknown, several stimuli, such as IL-4 [24], IL-10 [25] and granulocyte-macrophage colony-stimulating factor [26], have been shown to stimulate the production of IL-1ra in vitro. These facts, together with recent reports describing the presence of these cytokines in IBD [3,5], suggest that these cytokines may in part influence IL-1ra release in vitro. It is of interest that the acute phase proteins, CRP and α 1-antitrypsin, are potent inducers of IL-1ra [27]. In the present study, we found a high correlation between CRP and IL-1ra levels, suggesting that such unique induction of this antagonist also occurs in vivo. Moreover, this suggests that increased circulating acute phase proteins contribute indirectly to systemic down-regulation of active inflammation, as suggested previously in animal models [28]. These results suggest that circulating IL-1ra limits inflammatory disease, however, such concentrations are probably insufficient to overwhelm the exaggerated IL-1 production since at least a 10,000-fold molar excess of IL-1ra is required to prevent the action of IL-1 in vivo [11].

In conclusion, the results of the present study suggest that the plasma IL-1ra level in patients with IBD may be a useful marker of disease activity.

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IL-1ra IN INTESTINAL INFLAMMATION

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