Increased Soluble Interleukin-1 Type II Receptor Concentrations in Postoperative Patients and in Patients With Sepsis Syndrome


Plasma interleukin-1 (IL-1) activity is modulated in part through the simultaneous appearance of several inhibitors of IL-1 action, including interleukin-1 receptor antagonist (IL-1ra) and the soluble IL-1 type II receptor (IL-1RII). However, little is known concerning the plasma appearance of these inhibitors in patients following operative trauma or those with sepsis syndrome. In the present report, plasma IL-1β, IL-1ra, and soluble IL-1RI and IL-1RII concentrations were evaluated in 118 patients with sepsis syndrome or after elective operative trauma. Plasma concentrations of IL-1ra increased significantly following elective operative repair of thoraco-abdominal and abdominal aortic aneurysms, and after bowel resection for inflammatory bowel disease, but did not increase after laparoscopic cholecystectomy.

 interleukin-1 (IL-1) is a proinflammatory cytokine that is produced by activated immune cells in response to inflammatory stimuli. Although IL-1 has been shown to have many beneficial effects on the host during a stress response, IL-1 may be harmful if produced in excessive amounts or for an extended period of time. In many animal models and in human subjects with sepsis, shock, or ischemia/reperfusion injury, elevated IL-1 levels have been reported, and are often associated with organ damage, morbidity and mortality.

IL-1α and β bind to two classes of cell-surface receptors. The type 1 IL-1 receptor (IL-1RI, p80) is present on most cells and transduces the intracellular signal necessary to induce a cellular response. The type II IL-1 receptor (IL-1RII, p68) is found mainly on neutrophils, monocytes, B cells, and bone marrow progenitor cells, and does not appear to transduce a signal intracellularly; it has been postulated to act as a 'decoy' receptor, binding IL-1α and β, and thus preventing IL-1 from binding to the type I receptor to initiate signal transduction. In addition, soluble IL-1RII, which represents the extracellular domain of the membrane-bound IL-1RII formed by proteolytic cleavage, has been reported.

IL-1ra levels were also elevated in patients with sepsis syndrome. In contrast, soluble IL-1RII levels were only increased in patients after operative repair of thoraco-abdominal aortic aneurysms and in sepsis syndrome, whereas concentrations were unaffected by the other more modest surgical procedures. Plasma IL-1RI concentrations decreased in all postoperative patients in the first 24 hours after surgery. We conclude that both plasma IL-1ra and soluble IL-1RII concentrations increase in sepsis and following some operative trauma. Less severe operative trauma increases the plasma concentration of only IL-1ra, whereas both IL-1ra and soluble IL-1RII are increased in patients with sepsis syndrome or following thoraco-abdominal aortic aneurysm repair.

From the Department of Surgery, University of Florida College of Medicine, Gainesville; the Department of Surgery, The Free University Hospital, Amsterdam, The Netherlands; Amgen, Inc, Boulder, CO; Syntex Research Laboratories, Palo Alto, CA; and Immunex Research and Development Corporation, Seattle, WA.

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Address reprint requests to Hester S.A. Oldenburg, MD, Department of Surgery, The Free University Hospital, Amsterdam, The Netherlands.

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obtained from all patients with sepsis syndrome participating in the multicenter Antril clinical trial at their respective institutions before blood sampling (the Antril trial was a randomized, double-blind, placebo-controlled study evaluating the effects of recombinant human IL-1 receptor antagonist [Antril; Amgen Inc, Boulder, CO] in the treatment of sepsis syndrome).16

Thirty-five patients undergoing elective operative procedures at either Shands Hospital at the University of Florida College of Medicine or The Free University Hospital of Amsterdam, The Netherlands, were evaluated: 9 undergoing elective infrarenal abdominal aortic aneurysm repair (AAA) (average age 66 [age range 43-77], male 8:female 1), 8 undergoing elective thoraco-abdominal aortic aneurysm repair (average age 70 [age range 55-84], male 6:female 2), 9 undergoing laparoscopic cholecystectomy (1 patient converted to open cholecystectomy) (average age 45 [age range 28-70], male 3:female 6), and 9 undergoing surgical resection for inflammatory bowel disease (4 for ulcerative colitis, 2 for Crohn’s disease, and 3 for diverticulitis) (average age 45 [age range 21-77], male 6:female 3).

In addition, blood samples were obtained from 83 hospitalized patients with sepsis syndrome who participated in the multicenter, phase III clinical trial of IL-1ra (Antril). The 83 patients studied were chosen randomly from the total 897 patients enrolled into the study, as has been reported elsewhere.16 Blood samples were obtained at the time of randomization into the study, immediately before drug infusion, but after the patients had met the clinical criteria of sepsis syndrome.

In postoperative patients, blood was obtained from arterial lines or from venipuncture beginning 1 hour before surgery and continuing at regular intervals (0, 2, 4, 6 to 12, 24, 48, and 72 hours) throughout the perioperative and postoperative periods for 72 hours.

All blood was collected in sterile glass tubes containing EDTA, chilled immediately, then centrifuged at 2,600 rpm for 10 minutes. The plasma portion was then removed, placed into sterile tubes, and stored at −70°C until analyses were performed.

**Analytical methods.** IL-1RII, IL-1RI, IL-1ra, IL-1β, and PLA2 concentrations were all measured by enzyme-linked immunosorbent assay (ELISA) developed within the laboratory. IL-1RII was measured using a monoclonal rat-antihuman IL-1RII antibody (hIL-1RII-M25; Immunex Corp, Seattle, WA) as the coating antibody at 2 μg/mL. Protein A-purified rabbit polyclonal antihuman IL-1RII primary antibodies were used at 1 μg/mL. A goat-antirabbit IgG conjugated to horseradish peroxidase (HRP) was used as the secondary antibody. Samples were diluted 1:5 before assay. The lower limit of detection of the assay is 41 pg/mL. There was no cross-reactivity with the type I IL-1 receptor, IL-1ra, or IL-1β.

IL-1RI concentrations were measured by ELISA, using a monoclonal rat-antihuman IL-1RI antibody (hIL-1RI-M1; Immunex Corp) as the coating antibody at 5 μg/mL, a protein-A purificated rabbit-antihuman IL-1RI polyclonal antibody (hIL-1RI-P2; Immunex Corp) as the primary antibody at 10 μg/mL, and a goat-antirabbit IgG conjugated to HRP as the secondary antibody. Samples were not diluted. The lower limit of detection of this assay is 41 pg/mL. There was no cross-reactivity with the type II IL-1 receptor, IL-1ra, or IL-1β.

IL-1ra and IL-1β were measured also by ELISA using methodologies described in detail previously.15 Very briefly, plates were coated with protein-G purified mouse monoclonal antibodies (2IL-1RA-H24 or hIL-1β-H6, provided by Dr John Kenney, Institute Louis Pasteur, Strasbourg, France) at concentrations of 10 μg/mL and 5 μg/mL, respectively. A biotinylated mouse monoclonal antibody against human IL-1β (hIL-1β-H67) at 2 μg/mL was used as the primary antibody, and samples were visualized with streptavidin-HRP. For IL-1ra, the primary antibody was a 1:50 dilution of a polyclonal rabbit anti-IL-1ra antiserum (rabbits #9117,9118, pooled 8/92 bleed).

Samples were visualized with an HRP-conjugated goat, antirabbit antiserum. For IL-1ra, plasma samples were diluted 1:5 and for IL-1β, samples were added neat. The lower limits of detection for the IL-1ra and IL-1β ELISAs were 61 and 11 pg/mL, respectively. Neither assay is affected by the presence of either the soluble IL-1 type I or type II receptor.

Secretory phospholipase A2 (sPLA2) levels were used as an indicator of the severity of the inflammatory insult. sPLA2 was measured by ELISA using a monoclonal antihuman PLA2 as the coating antibody, a protein-A purified rabbit polyclonal antihuman PLA2 as the primary antibody, and a goat-antirabbit IgG-HRP as the secondary antibody (all reagents provided by Dr Robert Crowl, Hoffmann-La Roche, Inc, Nutley, NJ). Samples were diluted 1:20 (controls and patients undergoing laparoscopic cholecystectomy, abdominal aneurysm repair, or bowel resection for inflammatory bowel disease), 1:200 (patients undergoing thoraco-abdominal aortic aneurysm repair), or 1:500 (septic patients). The lower limit of detection of this assay on diluted samples is 0.6 ng/mL.

Data were analyzed by one-way analysis of variance (ANOVA). Differences in cytokine and shed receptor concentrations from baseline were assessed using Duncan’s multiple range test following ANOVA. In all cases, a two-tailed test and 95% confidence intervals were used.

**RESULTS**

**Thoraco-abdominal aneurysm repair.** Plasma concentrations of IL-1RII more than doubled postoperatively (P < .05). Concentrations peaked at 4 hours and decreased thereafter, although levels remained elevated for at least 72 hours postoperatively. Similarly, plasma levels of IL-1ra in these patients increased more than 30-fold (P < .05) over preoperative levels. However, concentrations peaked earlier than for IL-1RII (at 2 hours) and concentrations remained near peak levels for at least 72 hours postoperatively. In these patients, plasma IL-1RI levels diminished postoperatively, and this decrease (86%) was significant at 2 and 4 hours (P < .05) (see Fig 6). Plasma IL-1β concentrations were undetectable before surgery but peaked at 169 pg/mL at 1 hour postoperatively (P < .05), then returned to preoperative levels within 6 hours postoperatively (Fig 1).

sPLA2 concentrations were elevated greater than 10-fold 24 hours postoperatively, when compared with baseline levels (P < .05). In fact, sPLA2 levels were statistically higher in this group of patients than in all others, with the exception of patients with sepsis syndrome (Table 1).

The average hospital stay for these patients was 41 days, average intensive care unit (ICU) stay was 26.9 days, and 2 of the 8 patients (25%) died during their hospital stay. The incidence of multisystem organ dysfunction in this patient population was 50% (4 of 8).

**Abdominal aortic aneurysm repair.** Postoperative plasma levels of IL-1RII were not significantly elevated above preoperative concentrations. However, a small but significant increase (53%, P < .05) in plasma IL-1ra concentrations was observed after abdominal aortic aneurysm repair, with a peak seen at 2 hours postoperatively (Fig 2). A significant decrease (49%, P < .05) was seen in plasma IL-1RI levels postoperatively through 6 hours. IL-1β was not detected in the circulation preoperatively or postoperatively. Consistent with a more modest inflammatory insult, sPLA2 concentra-
IL-1ra concentrations increased significantly but modestly postoperatively (Fig 3). sPLA2 concentrations increased 400-fold over preoperative levels at 24 hours postoperatively. The average ICU stay was 0.3 days, with no postoperative mortalities.

Similar to the response after thoraco-abdominal aortic aneurysm repair. Samples were obtained at 0, 1, 2, 4, 6, 24, 48, and 72 hours following operative procedure and were screened by ELISA. Data were analyzed by one-way Analysis of Variance and Duncan’s multiple-range test. Asterisks indicate statistically significant differences from baseline (time 0), P < .05. Surgery resulted in the significant appearance of IL-1RII, IL-1ra, and IL-1β. Baseline plasma concentrations (pg/mL): IL-1RII 7,316 ± 2,693, IL-1ra 5,080 ± 731, IL-1β 0.0 ± 0.0.

The average hospital stay for these patients was 11.4 days, and there was no postoperative mortality. Patients with sepsis syndrome. Plasma measurements were obtained when the patients qualified for entry into the multicenter Antril trial, and before drug infusion. All patients met the criteria of sepsis syndrome, as determined by the clinical evaluation committee and summarized in the report of Fisher et al.16 Plasma soluble IL-1RII and IL-1ra concentrations were significantly elevated compared with baseline levels from preoperative patients in the four groups, and even peak measurements from the other patient populations (Fig 5A and B). The only exception was peak IL-1ra levels that were higher in those patients undergoing thoraco-abdominal aortic aneurysm repair than those with sepsis syndrome. However, IL-1β was not detected in any of the 83 septic pathways.

**Table 1. Peak Plasma Concentrations From Patients Undergoing Operative Procedures and From Patients With Sepsis Syndrome at Admission**

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>IL-1ra (pg/mL)</th>
<th>IL-1RII (pg/mL)</th>
<th>IL-1β (pg/mL)</th>
<th>IL-1RI (pg/mL)</th>
<th>PLA2 (ng/mL)</th>
</tr>
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<tbody>
<tr>
<td>TAAA</td>
<td>174,282 ± 58,068</td>
<td>16,048 ± 3,870</td>
<td>169 ± 77</td>
<td>3,415 ± 859</td>
<td>750 ± 449</td>
</tr>
<tr>
<td>AAA</td>
<td>10,710 ± 1,486</td>
<td>4,862 ± 987*</td>
<td>24 ± 20</td>
<td>1,401 ± 241*</td>
<td>93 ± 59</td>
</tr>
<tr>
<td>IBD</td>
<td>17,568 ± 5,056</td>
<td>6,711 ± 983*</td>
<td>10 ± 7</td>
<td>2,293 ± 1,235</td>
<td>255 ± 110</td>
</tr>
<tr>
<td>Lap Chole</td>
<td>4,129 ± 421</td>
<td>6,823 ± 1,639</td>
<td>0 ± 0*</td>
<td>1,210 ± 126*</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>Sepsis syndrome</td>
<td>31,324 ± 6,497</td>
<td>34,807 ± 6,084</td>
<td>0 ± 0*</td>
<td>2,018 ± 206</td>
<td>931 ± 409</td>
</tr>
</tbody>
</table>

**Abbreviations:** TAAA, thoraco-abdominal aortic aneurysm repair; AAA, abdominal aortic aneurysm repair; IBD, surgical resection for inflammatory bowel disease; Lap Chole, laparoscopic cholecystectomy.

* Peak values occurred at preoperative sampling time point.
Elevated IL-1RII levels

Patients. sPLA2 levels were also markedly elevated, with no significant difference when compared with those seen in thoraco-abdominal aneurysm repair.

Discussion

Excessive endogenous production of IL-1 that often results from overwhelming gram-negative bacteremia or endotoxicosis can often lead to shock and mortality. Blood-derived IL-1 can increase vascular permeability with pulmonary congestion, induce lung neutrophil infiltration and hemorrhage, and eventually cause myocardial suppression, shock, multi-organ system failure, and death, in part through the increased release of vasoactive mediators such as PGE2, PGH2, platelet-activating factor, sPLA2, and nitric oxide. Waage et al12 and Girardin et al14 reported that elevated plasma concentrations of IL-1 are associated with adverse outcome from meningococcal and systemic purpura infections.

Conversely, an endogenous IL-1 response is often essential to a successful outcome from bacterial and fungal infections. For example, we reported that blocking an endogenous IL-1 response in mice increased susceptibility to Listeria and Pneumocystis carinii infections.20,21 Van der Meer et al22-24 have even proposed administering IL-1 to increase antimicrobial resistance in normal and neutropenic animals.

Therefore, it is not surprising that given both the beneficial and adverse effects of IL-1 on the host, its activity in the blood would be tightly regulated. The host has developed several endogenous mechanisms to inhibit the actions of excessive IL-1 production, particularly when it appears in the systemic circulation. Two of these include the release of IL-1ra and the shedding of the IL-1RII. IL-1ra is a competitive inhibitor of IL-1 that binds to the type I IL-1 receptor with nearly equal affinity as IL-1β, but with no agonist activity. In several human studies, elevated plasma concentrations of IL-1ra have been noted after stimulation with endotoxin or bacteria, or in critically ill patients.14-15 The present studies can confirm that IL-1ra levels are increased in patients with sepsis syndrome (Fig 5B) and that these levels increase after most operative procedures (Figs 1 through 4).

Furthermore, the magnitude of the increase in IL-1ra appears to be related to the severity of the inflammatory insult, when sPLA2 levels are used to judge its magnitude. IL-1ra concentrations increased postoperatively in all patients with the exception of those following laparoscopic cholecystectomy. In patients following laparoscopic cholecystectomy, neither IL-1ra nor sPLA2 concentrations increased, a finding that is consistent with the very brief hospital stay (48 hours), and the observations of Jakeways et al25 that other proinflammatory cytokines are only minimally affected by this laparoscopic procedure. Vadas et al26,27 and Rintala and Nevalainen28 have argued that not only are sPLA2 concentrations prognostic for morbidity in critically ill patients with sepsis syndrome, but elevated concentrations in themselves may contribute to morbidity by activating prostanoid and platelet activating factor biosynthesis. Concentrations of sPLA2 also predict the incidence of pulmonary failure in patients with multiple injuries.29

The type II IL-1 receptor (IL-1RII) has no signal transduction function and has been postulated to be a 'decoy' receptor, binding IL-1 and thus preventing IL-1 from binding to the type I IL-1 receptor to produce an intracellular signal.3 Soluble IL-1 receptors representing the extracellular portion of IL-1RII generated by proteolytic cleavage have been noted previously in the circulation of normal subjects,30 in the synovia of patients with rheumatoid arthritis,31 and in supernatants from stimulated blood neutrophils.32 Recently, elevated concentrations in the plasma of septic patients have been reported.33

These data show, for the first time, evidence of increased plasma IL-1RII concentrations in patients following surgical
repair of thoraco-abdominal aortic aneurysms in addition to patients with sepsis syndrome. The repair of thoraco-abdominal aortic aneurysms is associated with significant ischemia and reperfusion injury of visceral organs secondary to transient (30- to 60-minute) suprarenal aortic cross-clamping, which is necessary to obtain proximal control of aortic blood flow during the procedure. In animal experiments, transient warm ischemia of the visceral arteries induces a proinflammatory cytokine response with lung, liver, and kidney injury. In these animal models, exaggerated IL-1 and tumor necrosis factor (TNFα) responses contribute to this pathophysiology, as pretreatment with inhibitors of IL-1 or TNF attenuates the subsequent neutrophil infiltration and organ damage.

A similar pattern is seen in this patient population. Plasma IL-1β concentrations were detected in 6 of 8 of the patients undergoing thoraco-abdominal aortic aneurysm repair and concentrations peaked at 169 pg/mL 2 hours after reperfusion. The frequency of detecting IL-1β in this population was significantly greater than was seen in patients with sepsis syndrome in the present report, or in the published literature, and is comparable to levels seen after infectious purpura in children. Similarly, thoraco-abdominal aortic aneurysm repair patients manifest postoperatively a systemic inflammatory response syndrome of comparable magnitude to that seen in patients with sepsis syndrome with 50% of them having evidence of multisystem organ dysfunction.

Thus, IL-1ra and soluble IL-1RII represent two distinct endogenous mechanisms by which the body modulates exaggerated levels of IL-1 in times of inflammatory challenge. Our data support the conclusion that in response to a modest systemic inflammatory stimulus, as occurs during most operative trauma, IL-1ra concentrations increase. In contrast, plasma IL-1RII levels are not as frequently increased. However, in response to more severe inflammatory stress, as occurs in sepsis syndrome or following visceral ischemia-reperfusion injury associated with thoraco-abdominal aortic aneurysm repair, increased release of soluble IL-1RII occurs in addition to IL-1ra.

It is noteworthy that IL-1RI concentrations decreased significantly in all of the postoperative patients in the immediate postoperative period, albeit the decreases were not significant in patients following intestinal resection for inflammatory bowel disease (Fig 6). This decline may reflect a decrease in the cell surface expression in response to IL-1β. This is consistent with previous in vitro findings by Ye et al., who showed that with only 2% to 15% of IL-1RI binding sites occupied by IL-1, surface expression of IL-1RI is reduced by 60% to 80%.

What remains unresolved is the functional significance of the increased appearance of IL-1ra and soluble IL-1RII in the plasma of septic patients and following thoraco-abdominal aortic aneurysm repair. Using a receptor binding assay, Giri et al has recently demonstrated that soluble IL-1RII has the highest affinity for IL-1β and can neutralize its bioactivity. In contrast, the affinity of soluble IL-1RII for IL-1ra and IL-1α is much less. This is not surprising, because IL-1β is the

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primary form of IL-1 found in the blood.15 Soluble IL-1RII binding to IL-1ra would essentially cancel the functioning of both inhibitors. Thus, soluble IL-1RII appears to function optimally for the neutralization of excess plasma IL-1β.

However, in the vast majority of septic patients where IL-1ra and shed IL-1RII are detected, there is no detectable IL-1β. Furthermore, in patients following thoraco-abdominal aortic aneurysm repair, IL-1β levels appear only transiently (lasting less than 2 hours) whereas IL-1ra and soluble IL-1RII concentrations remain elevated for periods exceeding 24 hours. This failure to detect IL-1β cannot be explained methodologically since the employed ELISA can detect IL-1β both in the free form and when bound to soluble IL-1RII (data not shown). In fact, Giri et al.10 reported using a binding assay in which sera from septic patients could bind and neutralize four times as much added IL-1β as could sera from healthy controls, suggesting that blood from critically ill patients had excess IL-1 inhibitory capacity. Thus, these patients at risk of multisystem organ failure, presumably from an exaggerated proinflammatory cytokine response, actually had increased quantities of natural IL-1 inhibitors in their plasma than did healthy controls.

The answer perhaps lies in the paracrine nature of IL-1 production. We and others have shown that tissue concentrations of IL-1 are markedly higher than they are in the plasma from animals and patients with hemorrhagic pneumonia17 or adult respiratory distress syndrome,38 respectively. The appearance of IL-1β in the circulation may result from excess tissue production that distributes into the plasma compartment. Since it has been well described that IL-1’s actions on the vascular endothelium lead to fluid and cellular extravasation, increased coagulability and excessive fibrin deposition in the capillary beds, all leading to hypotension and inadequate microperfusion,16 the presence of elevated plasma levels of IL-1ra and soluble IL-1RII may be one mechanism by which the host reduces the likelihood of systemic vascular responses to local tissue IL-1 production. If indeed this is the case, then shedding of the IL-1RII and increased release of IL-1ra may play important roles in downregulating the frequency with which local IL-1 production leads to a systemic inflammatory response.

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Increased soluble interleukin-1 type II receptor concentrations in postoperative patients and in patients with sepsis syndrome

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