Inflammatory mediators in human acute pancreatitis: clinical and pathophysiological implications

J Mayer, B Rau, F Gansauge, H G Beger

Abstract

Background—The time course and relationship between circulating and local cytokine concentrations, pancreatic inflammation, and organ dysfunction in acute pancreatitis are largely unknown.

Patients and methods—In a prospective clinical study, we measured the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, and IL-8, the anti-inflammatory cytokine IL-10, interleukin 1β receptor antagonist (IL-1RA), and the soluble IL-2 receptor (sIL-2R), and correlated our findings with organ and systemic complications in acute pancreatitis. In 51 patients with acute pancreatitis admitted within 72 hours after the onset of symptoms, these parameters were measured daily for seven days. In addition, 33 aspirates from ascites and the lesser sac were measured.

Results—Sixteen patients had mild acute pancreatitis (AP) and 35 severe AP (Atlanta classification); 18 patients developed systemic complications requiring treatment. All mediators were increased in AP, sIL-2R, IL-10, and IL-6 were significantly elevated in patients with distant organ failure. An imbalance in IL-1β/IL-1RA was found in severe AP and pulmonary failure. Peak serum sIL-2R predicted lethal outcome and IL-1RA was an early marker of severity. IL-6 was the best prognostic parameter for pulmonary failure.

Conclusion—Our results suggest that local mediator release, with a probable IL-1β/IL-1RA imbalance in severe cases, is followed by the systemic appearance of pro- and anti-inflammatory mediators. The pattern of local and systemic mediators in complicated AP suggests a role for systemic lymphocyte activation (triggered by local release of mediators) in distant organ complications in severe AP.

Key words: pancreatitis; cytokines; lymphocyte activation; pancreatic necrosis; organ complications

Morbidity and mortality in acute pancreatitis (AP) is largely determined by distant organ failure in severe attacks. These systemic manifestations of a disease initially limited to the pancreas are thought to be mediated by a variety of pro- and anti-inflammatory mediators released from the pancreas and various other sources during the course of the disease. Studies on AP have demonstrated that these mediators are produced in a variety of tissues in a predictable sequence, initiated by local release of proinflammatory mediators such as interleukin (IL)-1β, IL-6, and IL-8, which induce a systemic inflammatory response reflected by increased levels of soluble interleukin 2 receptor (sIL-2R), neopterin, or tumour necrosis factor α (TNF-α). This results in inflammatory infiltration of distant organs with multiorgan failure and death.

The systemic inflammatory response is kept at bay by local and systemic release of anti-inflammatory mediators such as interleukin 1β receptor antagonist (IL-1RA) and IL-10 which were shown to reduce the severity of pancreatitis and pancreatitis associated organ failure.

These observations demonstrate the potential of immunomodulation in preventing pancreatitis associated organ failure.

However, little is known of the relationship between the clinical course of AP in humans and the dynamics of the major cytokines, both locally and in the systemic circulation, in the presence or absence of distant organ complications. Therefore, we examined the relationship between local and systemic mediators in early AP in a prospective clinical trial.

Patients and methods

DEFINITIONS

Mild acute pancreatitis (MAP) was defined according to the Atlanta classification as confirmed acute pancreatitis without development of one or more major local or systemic complications caused by pancreatitis. Severe acute pancreatitis (SAP) was defined as AP associated with one or more major local or systemic complications caused by pancreatitis. Local complications include pancreatic necrosis, acute pancreatic fluid collection, pancreatic pseudocyst, or pancreatic abscess. Systemic complications include respiratory failure (pO2 <60 mm Hg requiring oxygen therapy for longer than 24 hours or mechanical ventilation), cardiocirculatory failure (systolic blood pressure <80 mm Hg for more than 15 minutes), renal failure (serum creatinine

Abbreviations used in this paper: IL, interleukin; IL-1RA, interleukin 1β receptor antagonist; sIL-2R, soluble interleukin 2 receptor; AP, acute pancreatitis; MAP, mild AP; SAP, severe AP; TNF-α, tumour necrosis factor α; SIRS, systemic inflammatory response syndrome; CRP, C reactive protein; LR, likelihood ratio; ERCP, endoscopic retrograde cholangiopancreatography.

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Table 1  Complications of mild and severe acute pancreatitis (AP)

<table>
<thead>
<tr>
<th>Necrosis</th>
<th>Pulmonary</th>
<th>Renal</th>
<th>Death</th>
<th>Sepsis</th>
<th>Shock</th>
<th>Infected necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild AP (n=16)</td>
<td>0</td>
<td>2 without ventilation</td>
<td>1 without dialysis</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Severe AP (n=35)</td>
<td>34</td>
<td>12 without ventilation, 18 with ventilation</td>
<td>8 without dialysis 6 with dialysis</td>
<td>13</td>
<td>11</td>
<td>7 without catecholamines 12 with catecholamines</td>
</tr>
</tbody>
</table>

>2 mg% in the absence of prior renal insufficiency), or the presence of gastrointestinal haemorrhage. Bacteria negative sepsis was defined as the systemic inflammatory response syndrome (SIRS) without positive blood culture according to the definitions of the ACCP/SCCM consensus conference committee.7 Sepsis was defined as blood culture positive SIRS.

PATIENTS
Patients with confirmed AP (clinical symptoms, amylase/lipase greater than three times the upper limit, and pancreatitis on ultrasound or computed tomography scan) who were admitted to our hospital as primary or secondary referrals within 72 hours after the onset of symptoms were prospectively entered into the study. The study was performed according to local ethics committee regulations and informed consent was obtained. Exclusion criteria were age <18 years, infection with hepatitis or human immunodeficiency virus, pregnancy, and refusal of consent. A total of 51 patients were included in the study; 16 suffered from mild AP (MAP) and 35 from severe AP (SAP) according to the Atlanta classification.6 Of the patients with MAP, seven were male and nine were female (median age 65 years (range 19–83)). The aetiology in MAP was biliary (n=10), alcoholic (n=4), post-endoscopic retrograde cholangiopancreatography (ERCP) (n=1), and idiopathic (n=1). Twenty four patients with SAP were male and 11 were female (median age 46 years (range 32–79)). The aetiology in SAP was alcoholic (n=20), biliary (n=11), post-ERCP (n=3), and idiopathic (n=1). Median Ranson scores were 7 for MAP and 6 for SAP, and median admission APACHE II scores were 9 for MAP and 12 for SAP. Both scores were significantly higher in SAP than in MAP (p<0.05).

All patients with mild AP suffered from interstitial pancreatitis; two of these patients developed pulmonary insufficiency but did not require mechanical ventilation. Of 35 patients with severe AP, 34 had pancreatic necrosis and one patient with interstitial pancreatitis suffered from renal failure. Thirteen patients with pancreatic necrosis died during their hospital stay, resulting in an overall hospital mortality of 25.5%. Of the patients who died, six (46%) suffered from infected necroses, while in the survivor group only three patients (15.8%) had bacterial infection. In severe AP, 19 patients suffered from cardiocirculatory failure (“shock”), 12 of whom were treated with intravenous catecholamines. Only two patients with pancreatic necrosis did not develop any systemic complications; 14 developed systemic complications but needed no intervention while 18 developed systemic complications requiring mechanical ventilation (n=18), dialysis (n=6), or surgical intervention (n=10) (table 1).

METHODS
The cytokines IL-1β, IL-6, IL-10, the antagonist IL-1RA, and the soluble IL-2 receptor (sIL-2R) were measured in serum at intervals using commercially available ELISA kits (DPC Biermann, Bad Nauheim, Germany and R&D System, Oxford, UK) on a standard ELISA reader according to the manufacturer’s instructions. IL-8 was measured by a chemiluminescent immunoassay using the Immulite automated Luminometer (DPC Biermann, Bad Nauheim, Germany). The cytokines were chosen based on experience obtained in previous studies. As markers for pancreatic and monocyte/macrophage cytokine release,2–4 the proinflammatory IL-1β and its receptor antagonist IL-1RA were chosen. TNF-α was not measured as it is unstable, difficult to measure in the clinical setting, and is expressed in the pancreas in a similar manner as IL-1β.5–11 As a marker of lymphocyte activation, we measured the soluble cleaved interleukin 2 receptor CD25, sIL-2R. IL-6 was determined as a lymphocyte activating cytokine and IL-8 as a neutrophil activating chemokine. IL-10 was

Table 2  Peak values of routine chemical parameters in serum in mild and severe acute pancreatitis (AP) (mean (SEM))

<table>
<thead>
<tr>
<th>Amylase (U/l)</th>
<th>Lipase (U/l)</th>
<th>LDH (U/l)</th>
<th>CRP (mg/dl)</th>
<th>WBC (10³/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe AP</td>
<td>1186 (769)</td>
<td>2330 (545)</td>
<td>616 (126)</td>
<td>321 (51)</td>
</tr>
<tr>
<td>Mild AP</td>
<td>1059 (420)</td>
<td>1844 (632)</td>
<td>243 (61)</td>
<td>160 (45)</td>
</tr>
<tr>
<td>p Value</td>
<td>NS</td>
<td>0.01</td>
<td>0.005</td>
<td>NS</td>
</tr>
</tbody>
</table>

LDH, lactate dehydrogenase; CRP, C reactive protein; WBC, white blood cell count.

Table 3  Peak values of mediators in ascites and lesser sac (local) compared with peak serum values in severe acute pancreatitis (mean (SEM))

<table>
<thead>
<tr>
<th>sIL-1RA (U/ml)</th>
<th>sIL-1β (pg/ml)</th>
<th>sIL-2R (pg/ml)</th>
<th>IL-6 (ng/ml)</th>
<th>IL-10 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local (n=24)</td>
<td>3614.8 (809)</td>
<td>448.8 (211)†</td>
<td>1297 (163)</td>
<td>1339 (149)†</td>
</tr>
<tr>
<td>Serum (n=35)</td>
<td>3616 (407)</td>
<td>4.88 (1.05)†</td>
<td>1440 (205)</td>
<td>666 (101)†</td>
</tr>
</tbody>
</table>

IL-1, interleukin; IL-1RA, interleukin 1β receptor antagonist; sIL-2R, soluble interleukin 2 receptor.
†Significant difference.
measured as a marker for Th2 lymphocyte activity.12

Routine laboratory parameters were determined in serum samples taken at the same time as samples for cytokine measurements (at the Department of Clinical Chemistry, University of Ulm, Germany). These parameters were serum concentrations of amylase, lipase, lactate dehydrogenase, C reactive protein (CRP), and total white blood cell count.

**SAMPLE COLLECTION AND MEASUREMENTS**

Blood samples were obtained on admission and at 24 hour intervals for the next six days of hospital treatment. Blood was collected as serum into EDTA tubes, immediately centrifuged at 3500 g for 10 minutes, aliquoted in 500 µl portions, and stored at −70°C. Additionally, 24 ascites and nine lesser sac samples from 20 patients with SAP were obtained by fine needle aspiration or at laparotomy, centrifuged at 3500 g for 10 minutes, and stored at −70°C for subsequent analysis.

**STATISTICAL ANALYSIS**

Statistical analysis was performed using the MedCalc statistical program (Schoonjans, Netherlands). All values are expressed as mean (95% confidence intervals) and were compared using the Mann-Whitney U test. Correlation in multiple regression analysis was considered statistically significant when p<0.05. Cut off values, sensitivity, specificity, and positive (+LR) and negative likelihood ratios (−LR) were determined by receiver operating characteristic analysis. A multiple regression analysis was performed comparing the independent variables pancreatic necrosis, sepsis, renal, cardio-circulatory and pulmonary failure, infected necrosis, and death.

**Results**

**ROUTINE PARAMETERS**

Table 2 shows the routine laboratory parameters in severe and mild AP. Amylase and lipase levels peaked on the first day but did not distinguish mild from severe attacks. CRP reached its highest value on day 3 and was significantly higher in SAP than in MAP (320 (102) v 160 (89) ng/ml; p<0.005). CRP and white blood count remained elevated throughout the observation period in SAP but not in MAP.

**Figure 1** Time course of the proinflammatory mediators (interleukins (IL)-1β, IL-6, and IL-8, and soluble interleukin 2 receptor (sIL-2R)) in serum in human acute pancreatitis. Values are median (interquartile range). SAP, severe acute pancreatitis; MAP, mild acute pancreatitis.

**Figure 2** Time course of the anti-inflammatory mediators (interleukin 10 (IL-10) and interleukin 1β receptor antagonist (IL-1RA)) in serum in human acute pancreatitis. Values are median (interquartile range). SAP, severe acute pancreatitis; MAP, mild acute pancreatitis.
Table 5 Peak serum concentrations of the mediators in survivors and non-survivors in acute pancreatitis (median (interquartile range))

<table>
<thead>
<tr>
<th>IL-1β (pg/ml)</th>
<th>IL-1RA (U/ml)</th>
<th>sIL-2R (pg/ml)</th>
<th>IL-6 (ng/ml)</th>
<th>IL-8 (pg/ml)</th>
<th>IL-10 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe AP (n=13)</td>
<td>8.25 (3.6-8.2)</td>
<td>4759 (3451-7000)</td>
<td>969 (574-2582)</td>
<td>722 (347-1680)</td>
<td>100 (73-158)</td>
</tr>
<tr>
<td>Mild AP (n=16)</td>
<td>2.87 (0.1-4.4)</td>
<td>1796 (1515-2425)</td>
<td>568 (373-819)</td>
<td>268 (107-443)</td>
<td>110 (59-678)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.05</td>
<td>0.01</td>
<td>0.005</td>
<td>0.006</td>
<td>NS</td>
</tr>
</tbody>
</table>

IL, interleukin; IL-1RA, interleukin 1 receptor antagonist; sIL-2R, soluble interleukin 2 receptor.

Table 6 Peak serum values of mediators in various clinical complications in acute pancreatitis (median (interquartile range))

<table>
<thead>
<tr>
<th>IL-1β (pg/ml)</th>
<th>IL-1RA (U/ml)</th>
<th>sIL-2R (pg/ml)</th>
<th>IL-6 (ng/ml)</th>
<th>IL-8 (pg/ml)</th>
<th>IL-10 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sepsis (n=40)</td>
<td>2.93 (0.5-5.7)</td>
<td>2751 (1717-6391)</td>
<td>727.3 (448-1077)</td>
<td>427.6 (223-1059)</td>
<td>114 (54-706)</td>
</tr>
<tr>
<td>Sepsis (n=11)</td>
<td>4.93 (4.1-8.5)</td>
<td>3825 (3306-6585)</td>
<td>1528.5 (792-2224)</td>
<td>867 (524-1507)</td>
<td>158 (121-390)</td>
</tr>
<tr>
<td>p Value</td>
<td>NS</td>
<td>NS</td>
<td>0.04</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>No pulmonary dysfunction (n=33)</td>
<td>2.8 (0.5-4.2)</td>
<td>2751.5 (1639-4176)</td>
<td>581.4 (428-776)</td>
<td>295 (219-429)</td>
<td>88.2 (41-1086)</td>
</tr>
<tr>
<td>Pulmonary failure (n=18)</td>
<td>7.0 (5.1-17.1)</td>
<td>3495.5 (3265-8260)</td>
<td>2224 (960-3205)</td>
<td>1760 (989-2071)</td>
<td>157 (95-337)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.004</td>
<td>0.004</td>
<td>0.001</td>
<td>0.0005</td>
<td>0.0006</td>
</tr>
<tr>
<td>No renal dysfunction (n=37)</td>
<td>3.47 (2.5-5.1)</td>
<td>3703 (2451-5453)</td>
<td>700 (504-971)</td>
<td>392 (232-693)</td>
<td>86.2 (65-164)</td>
</tr>
<tr>
<td>Renal failure (n=14)</td>
<td>7.1 (5.9-10.4)</td>
<td>8669 (3929-14508)</td>
<td>3259 (2620-4261)</td>
<td>1670 (1505-1780)</td>
<td>155 (152-158)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.03</td>
<td>0.03</td>
<td>0.001</td>
<td>0.003</td>
<td>NS</td>
</tr>
</tbody>
</table>

IL, interleukin; IL-1RA, interleukin 1 receptor antagonist; sIL-2R, soluble interleukin 2 receptor.

Discussion

In the clinical setting, early diagnosis and, if possible, assessment of the prognosis of AP is of major interest for the clinician. In previous clinical studies, pancreatic enzyme release was found to be a good diagnostic parameter in AP.

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But while amylase and lipase are generally accepted as diagnostic for AP, they are not prognostic of its severity.13 CRP has been established as a prognostic variable in human AP.14 In our prospective clinical study, we confirmed both the diagnostic value of serum amylase/lipase and the prognostic value of peak serum CRP. However, serum concentrations of CRP reached their peak only three days after the onset of symptoms, which considerably diminishes its clinical usefulness in early AP. In this respect, peak serum concentrations of IL-6 was a much earlier prognostic parameter in assessing the severity of AP. Previous studies have established the role of IL-6 as an early marker of severity.15-16 In the clinical setting, IL-6 increases earlier and is a more sensitive marker of severity than CRP.20 Within the first 24 hours after disease onset, IL-6 is superior to β, microglobulin and comparable with IL-8.21 Our findings support the combination of IL-6 with lipase, as recently suggested for the diagnosis and prognosis of AP in the clinical setting.22

IL-8, a neutrophil activating chemokine, has been found to correlate with disease severity in human AP.23-24 In determining the severity of AP, systemic complications have been found to be associated with a significant increase in monocyctic secretion of TNF-α, IL-6, and IL-8 in patients with complicated AP.25 The same has been found for the release of IL-6, IL-8, and TNF-α by peripheral blood mononuclear cells in patients with severe AP.26 In a clinical study, the role of serum IL-8 in predicting lethal AP was confirmed in another study by our group.27 In our study, peak concentrations of serum IL-8 were increased in lethal AP but peak values within the first seven days after the onset of symptoms were not significantly different in severe or complicated AP.

The release of the cytokine IL-1β and IL-6 from inflamed pancreatic tissue has been linked to the development of distant organ dysfunction.15 Furthermore, increased pancreatic expression of IL-1β was seen in experimental AP,28 inactivation of the IL-1 converting enzyme ICE has been found to result in milder pancreatitis and improved survival in experimental pancreatitis.29 Supporting these observations, we found that IL-1β was about 100-fold increased locally compared with peak systemic concentrations and local concentrations of IL-6 were twice as high as peak serum concentrations in severe AP. Peak serum concentrations of IL-1β and IL-6 were significantly elevated in severe and lethal AP as well as in cases complicated by pulmonary and renal failure. In view of the aforementioned experimental studies, our study provides further support for local proinflammatory cytokine release in the development of distant organ complications.

As there is negative feedback between IL-1β and IL-1RA,1 the IL-1 receptor antagonist (IL-1RA) may attenuate the severity of AP. Reduced pulmonary injury in rodent necrotizing pancreatitis treated with IL-1RA28 and a beneficial effect of IL-1RA on survival and necrosis in experimental AP29-30 have been described. Increased serum IL-1RA has been found in human AP, and in pancreatitis and sepsis, increased IL-1RA was associated with severity and multiorgan dysfunction.31 A lower IL-1β/IL-1RA ratio was found in AP complicated by sepsis and infected necrosis32 which gave rise to the notion that an imbalance in IL-1β/IL-1RA is involved in the pathogenesis of complicated AP. In our study in human AP, IL-1RA distinguished between mild and severe attacks during the first 48 hours after the onset of symptoms with high sensitivity and specificity. Furthermore, we found that, contrary to IL-1β, local IL-1RA concentrations in severe AP were not higher than peak serum values. While peak serum concentrations of IL-1β were significantly increased in AP complicated by pulmonary failure and during lethal courses of the disease, the receptor antagonist IL-1RA was not significantly elevated. The relationship between local and systemic concentrations of IL-1β and IL-1RA in complications in human AP may help to substantiate the concept of a pro- and anti-inflammatory imbalance in severe AP.

A pathophysiological role for IL-6 in the acute phase response comes from the broad proinflammatory action on various other cells and induction of the production of acute phase proteins.33-34 In experimental AP, IL-6 has been shown to be associated with distant organ complications35 and increased IL-6 has been linked to adult respiratory distress syndrome.36 In isolated peripheral blood monocytes, increased IL-6 release was associated with systemic complications.37-38 Similarly, in our study in human AP, IL-6 was a sensitive and specific marker of development of pulmonary failure and peak serum concentrations were increased in severe and lethal AP as well as in pulmonary and renal failure.

The anti-inflammatory cytokine IL-10 is a marker of Th2 lymphocyte activity.22 Before studies on IL-10 in human AP were conducted, a number of experimental studies on the potential of IL-10 in reducing necrosis and mortality in AP were published.4-5 35 36 37 Underlining the potential anti-inflammatory effect, lower concentrations of serum IL-10 were described in mild early AP in humans.38 However, serum levels of IL-10 in severe AP were found to be increased in a recent study.39 Two other studies in human AP also found higher IL-10 levels in severe pancreatitis, serum IL-10 remained elevated for longer than mild pancreatitis, and were positively correlated with increased IL-6 and mortality.15-16 In our study we confirmed the view of IL-10 as an early marker of severity; serum IL-10 levels were increased in severe and lethal pancreatitis as well as in sepsis, and pulmonary and renal failure. Together with the soluble IL-2 receptor (sIL-2R), serum IL-10 was the only parameter that was significantly increased in sepsis. This may be indicative of the role of T lymphocyte activation in the host defence against bacteria.

An increase in serum levels of sIL-2R and sCD8 combined with a decrease in serum sCD4 in AP compared with healthy individuals suggests early lymphocyte activation in AP.40

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Reduced circulating levels of CD4 positive lymphocytes in AP, which correlated with increased endotoxin and IL-6, confirm this observation. An imbalance of the macrophage-lymphocyte system with inadequately high lymphocyte activation in severe AP (reflected by increased levels of interferon γ which is cosecreted with IL-2 by Th1 cells) has been suggested previously. Increased levels of sIL-2R in severe AP have been found in human pancreatitis and increased expression of IL-2 receptor on lymphocytes as a marker of systemic lymphocyte activation in severe AP has been reported. In our clinical study, we confirmed the important role of sIL-2R as a marker for severe AP, especially in AP complicated by pulmonary or renal failure, or sepsis and during lethal courses of the disease. In fact, peak serum sIL-2R levels within the first seven days of the disease was a sensitive predictor of lethal outcome.

In experimental AP, systemic lymphocyte activation has been linked to the development of distant organ complications. The development of pancreatitis associated distant organ failure, particularly pulmonary failure, has been found to be reduced in the absence of functional lymphocytes. Depletion of circulating CD3+ lymphocytes or pancreatectomy depressed activation in experimental AP has been found to reduce systemic complications. In our clinical study, we identified the specific role of the proinflammatory cytokine IL-6, the lymphocyte activation marker sIL-2R, and the TH2 cytokine IL-10 in AP with complications. While this does not represent definite proof, together with a large body of evidence from experimental studies it allows interesting interpretations on the role of systemic lymphocyte activation in human AP. However, as no further conclusions can be drawn from this present study, more studies are needed to identify which lymphocyte subsets are involved and their time course of activation. Definitive knowledge of the immunological phenomenon may lead to the development of immunomodulatory treatments in severe AP.

Conflict of interest statement. T Simon, M Hoover, H Quan and J Bolognese are employees of Merck & Co Inc and potentially sored in part by a grant from the interdisciplinary centre for immunomodulatory treatments in severe AP.


