ORIGINAL ARTICLE

Different profiles of cytokine expression during mild and severe acute pancreatitis

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Abstract

AIM: To study secretion patterns of pro- and anti-inflammatory cytokines, and activation of various cellular subsets of leukocytes in peripheral blood.

METHODS: We have conducted a prospective observational study. One hundred and eight patients with a diagnosis of acute pancreatitis and onset of the disease within last 72 h were included in this study. The mRNA expression of 25 different types of cytokines in white blood cells was determined by quantitative real time polymerase chain reaction. Levels of 8 different cytokines in blood serum were measured by enzyme linked immunosorbent assay. Clinical data and cytokine expression results were subjected to statistical analysis.

RESULTS: Severe and necrotizing acute pancreatitis (AP) is characterized by the significant depletion of circulating lymphocytes. Severe acute pancreatitis is associated with a typical systemic inflammatory response syndrome and over-expression of pro-inflammatory cytokines [interleukin (IL)-6, IL-8, macrophage migration inhibitory factor (MIF)]. Serum IL-6 and MIF concentrations are the best discriminators of severe and necrotizing AP as well as possible fatal outcome during the early course of the disease.

CONCLUSION: Deregulation of cellular immune system is a key event leading to severe and necrotizing AP. IL-6 and MIF could be used as early predictors of complications.

INTRODUCTION

Incidence of acute pancreatitis (AP) is about 30-40 cases per 100 000 individuals, and it carries an overall mortality

rate of 10%-15%[1-3]. Mortality of patients with severe acute pancreatitis approaches 30%-40%[4-6]. Pancreatic necrosis develops early in the course of the disease and usually is well established by 96 h after the onset of clinical symptoms[7,8]. Both the risk of multiorgan failure (MOF) and infectious complications appear to be related to the degree of pancreatic necrosis[9]. If patients survive the critical early stages of systemic inflammatory response, development of septic complications is a major determinant of survival. Infected pancreatic necrosis and sepsis-related multiple organ failure account for up to 40%-50% of all deaths among AP patients[10-13].

At present it is widely accepted that the premature activation of digestive enzymes within pancreatic acinar cells is an initiating event that leads to the autodigestion of pancreas[14-16]. Once the disease process is initiated, common inflammatory and repair pathways are invoked. There is a local inflammatory reaction at the site of injury; if marked, this leads to a systemic inflammatory response syndrome (SIRS). An imbalance between the early SIRS, and the later compensatory counter-inflammatory response (CARS), and development of MOF are considered to be the primary causes of morbidity and mortality in severe acute pancreatitis. Excessive leukocyte activation (including neutrophils and monocyte-macrophage lineage) with cytokinemia plays a critical role in the pathogenesis of pancreatitis and even more so, of the subsequent inflammatory response[14-17]. It has been hypothesized that fatal pancreatitis is a consequence of abnormal phagocytic leukocyte hyperstimulation due to deregulation in T- and B-lymphocyte activation. However, the role of lymphocyte activation and its relation to the severity of disease in humans is still poorly understood[18-21]. Another important drawback, in our opinion, is that the majority of information about the alterations of the immune system during the AP comes from *in vitro* and *in vivo* studies, and therefore it is not always directly applicable and relevant to the clinical situation in human acute pancreatitis.

Several methods for estimating severity of acute pancreatitis are widely used today and include APACHE II, Imrie and Ranson scores, the CT scoring system, and measurement of C-reactive protein and a number of laboratory markers[22-28]. Most of these multifactorial scores and laboratory tests are very good at identifying the critical situation, when MOF or local complication supervene, however, none can accurately predict the disease severity, development of pancreatic necrosis and/or possible fatal outcome during the first hours of hospitalization.

Thus, the aims of this study have been to investigate the secretion patterns of pro- and anti-inflammatory cytokines, and to estimate the activation of various cellular subsets of leukocytes (including lymphocytes, neutrophils, and monocyte-macrophage lineage) in peripheral blood of patients with severe and mild AP. Another goal of this study was to identify the serum soluble molecules that are associated with the development of severe acute pancreatitis, and which could be used as early markers to assess the local (pancreatic necrosis) and systemic complications (SIRS, MOF) later in the course of the disease.

MATERIALS AND METHODS

### Study design and patient population

We have conducted a prospective observational study in the period between June 2005 and December 2007. All patients admitted to the Department of Surgery at Kaunas University of Medicine Hospital (Lithuania) with a diagnosis of acute pancreatitis and onset of the disease within last 72 h were included in this study (*n* = 108). The Regional Ethics Committee approved the study (protocols No. BE-2-47 and P1-113/2005) and all the patients provided written informed consent.

The diagnosis was established on the basis of acute abdominal pain, at least 3-fold elevated levels of serum amylase and typical radiological findings. A contrast-enhanced CT scan was performed on days 4 to 7 after onset of the disease to demonstrate the presence of pancreatic necrosis. According to the clinical course and clinical severity scores (APACHE II > 7; Imrie-Glasgow > 2; MODS > 2), patients were stratified into mild and severe acute pancreatitis groups. Clinical data relating to the severity of disease, development of organ dysfunction and/or septic complications were prospectively collected in a standardized fashion. Patients with underlying chronic pancreatitis and patients with acute pancreatitis referred to our hospital from other institutions after management for more than 3 d were excluded from this study. Age- and sex-matched healthy subjects (*n* = 18) without previous medical history were enrolled as controls.

Peripheral blood samples from patients were drawn on admission to the hospital and, after centrifugation, samples were stored at -80°C until analysis. The blood samples of control group underwent a similar process. Blood sample analysis was uniformly performed at the Laboratory for Molecular Research of Pancreas, Department of Surgery, Heidelberg University (Germany). The mRNA expression of 25 different types of cytokines in white blood cells (WBCs) was determined by quantitative real time polymerase chain reaction (QRT-PCR). Levels of 8 different cytokines in blood serum were measured by enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Sigma-Aldrich GmbH, Steinheim, Germany).

Statistical analysis

Clinical data and cytokine expression results were used for statistical analysis. Statistical analysis was performed using SPSS® for Windows release 14.0 (SPSS, Chicago, Illinois, USA). Discriminant function analysis was used to determine differences between the groups. The diagnostic performance of a test, with a particular cut-off value, was established using Receiver Operating Characteristic (ROC) curve analysis. The data are presented as mean ± SE or median. For comparison between groups, the Mann-Whitney test or Student's *t* test were employed where appropriate. Results with $P < 0.05$ were considered statistically significant.
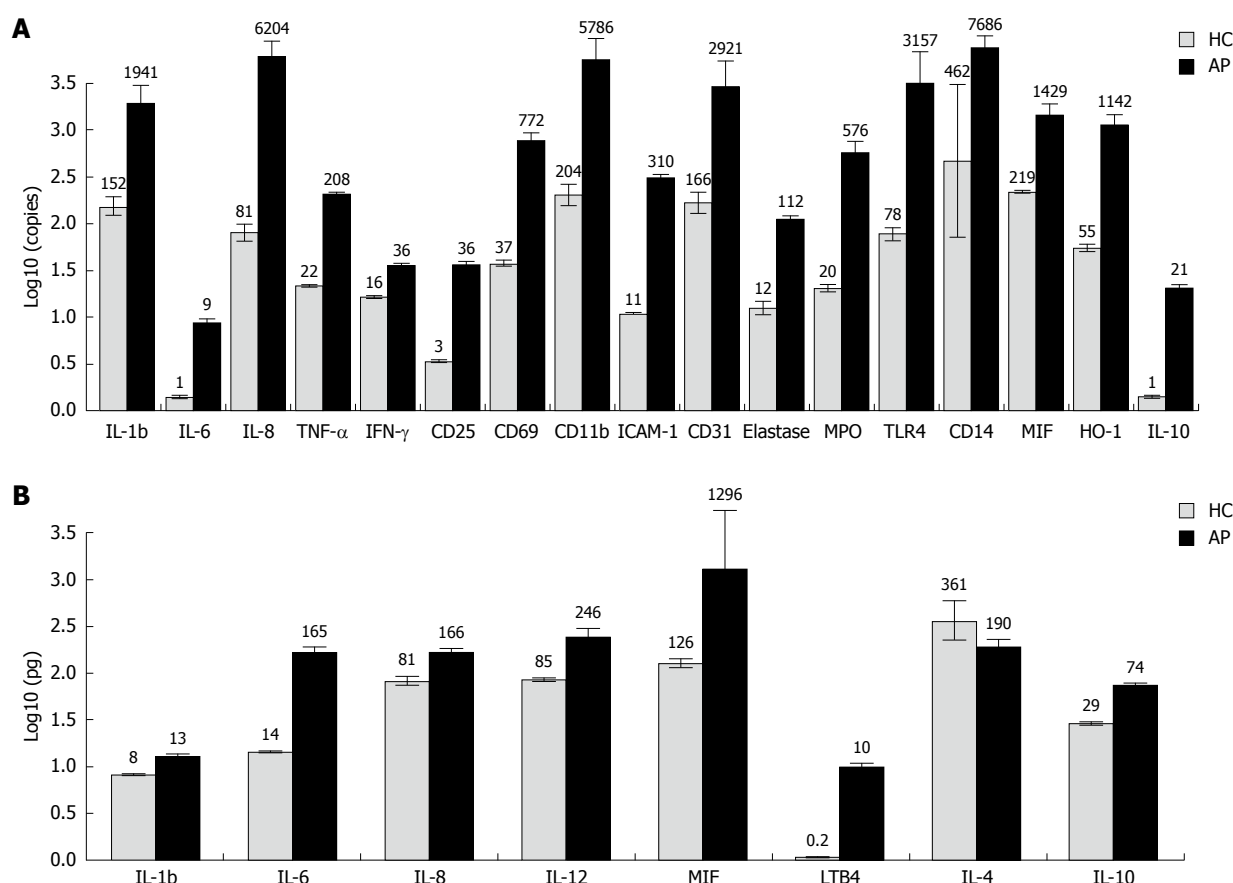
Figure 1 Cytokine expression at mRNA and protein level in peripheral blood of healthy subjects and acute pancreatitis (AP) patients. A: Gene expression in peripheral blood leukocytes of healthy subjects and AP patients. Gene expression at mRNA level of 25 different cytokines, adhesion molecules, lymphocyte activation markers, and other biologically active substances known to be linked to the inflammatory response were assessed by quantitative real time polymerase chain reaction (QRT-PCR) in peripheral blood leukocytes of AP patients and healthy controls. A median 18-fold (range: 2-77) increase in mRNA expression of pro-inflammatory molecules was observed in the AP group in comparison to healthy control subjects (HC). Activation of the anti-inflammatory system and some cytoprotective molecules was also notable in the AP patient group. Cytokine mRNA expression levels with statistically significant differences between AP and healthy control groups are shown; B: Cytokine concentration in blood serum of healthy controls and AP patients. Expression of eight different soluble molecules at protein level was assessed in serum of AP patients and healthy subjects using the enzyme linked immunosorbent assay (ELISA) method. A median 3-fold (range: 2-59) increase in protein expression of pro-inflammatory molecules was observed in the AP group when compared to HC. Cytokine expression levels with statistically significant differences between AP and healthy control groups are shown. IL: Interleukin; TNF-α: Tumor necrosis factor-α; IFN-γ: Interferon-γ; MPO: Myeloperoxidase; MIF: Macrophage migration inhibitory factor.

RESULTS

One hundred and eight patients (55 male and 53 female, mean age 54.1 ± 16.7 years) diagnosed with AP were included in the study. The main etiological factors of AP were alcohol (42.6%), high-fat diet (25.9%) and biliary stones (25.0%). Idiopathic pancreatitis was diagnosed in 7 cases (6.5%). Severe acute pancreatitis (APACHE II > 7) comprised 44.4% of all cases. Necrotizing AP was detected by contrast-enhanced CT in 52 patients (48.1%), and high volume (> 30%) necrosis was present in 34 cases (31.3%). Multiple organ failure (> 2 systems) developed in 18 cases (16.7%). Overall mortality in our follow-up group was 12.9%; 9 patients (8.3%) died within first 2 wk of the disease and 5 patients (4.6%) later in the course of the disease. Eighteen healthy subjects (10 male and 8 female, mean age 40.1 ± 19.8 years) were studied as controls.

Gene expression at mRNA level of 25 different cytokines, adhesion molecules, lymphocyte activation markers, and other molecules known to be linked to the inflammatory response were assessed in peripheral blood leukocytes of AP patients and healthy controls. Results showed a typical pro-inflammatory cytokine profile in AP patients with an activation of different subsets of leukocytes (lymphocytes, monocyte-macrophage lineage and neutrophils). Activation of anti-inflammatory system and some cytoprotective molecules was also notable in the AP patient group (Figure 1A). A median 18-fold (range: 2-77) increase in mRNA expression of pro-inflammatory molecules was observed in the AP group in comparison to healthy controls. Expression of eight different soluble molecules at protein level was also assessed in peripheral blood serum of AP patients and healthy subjects using the ELISA method. Analysis revealed a typical over-expression of pro-inflammatory cytokines and altered expression of anti-inflammatory cytokines in AP patients compared to the healthy volunteers (Figure 1B). A median 3-fold (range: 2-59) increase in protein expression of pro-inflammatory molecules was observed in the AP group in comparison to healthy controls. Since many of these changes have been shown in other animal and clinical studies, this served as a

Table 1 Differences in clinical course of mild and severe acute pancreatitis (mean ± SD/count)

Clinical severity scores and patient characteristics	Mild AP	Severe AP	P
Apache Ⅱ (score)	3.6 ± 1.9	11.6 ± 4.7	< 0.01
Imrie-Glasgow (score)	2.2 ± 1.2	4.4 ± 1.5	< 0.01
MODS (score)	1.1 ± 1.3	4.3 ± 3.4	< 0.01
Necrotizing AP (count)	16	36	< 0.01
C-reactive protein (mg/L)	91.8 ± 18.7	152.9 ± 21.5	< 0.01
Necrosis volume > 30% (count)	8	26	< 0.01
Infectious complications (count)[1]	2	12	< 0.01
Presence of MOF (count)	0	18	< 0.01
Need for surgery (count)[2]	1	5	0.06
ICU stay (d)	1.0 ± 1.0	9.0 ± 2.0	< 0.05
Total hospital stay (d)	12.0 ± 1.0	19.0 ± 3.0	< 0.05
Number of early deaths (< 2 wk)	0	9	< 0.01
Number of late deaths (> 2 wk)	0	5	< 0.01

[1]Infectious complications: proven sepsis, infected pancreatic necrosis, lung and urinary infection; [2]Need for surgery: fasciotomy for intraabdominal compartment syndrome and/or necrosectomy. AP: Acute pancreatitis; MOF: Multiple organ failure; ICU: Intensive care unit.

control and proved that our methods function well.

For the next step of statistical analysis, AP patients were stratified into groups according to the severity of the disease based on presence of systemic (development of MOF) and local (pancreatic necrosis > 30%) complications, since these are the factors know to be associated with a poor outcome. Groups were formed on the basis of radiological findings on contrast-enhanced CT and APACHE Ⅱ clinical score, where all patients with score > 7 were ascribed to the severe acute pancreatitis (SAP) group. Both SAP and necrotizing AP were associated with higher incidence of MOF, higher mortality and prolonged ICU and overall hospital stay. Whereas mild AP patients showed 0% mortality, overall mortality rate among SAP patients was 27% ($P < 0.01$) and early mortality (during first two weeks from the onset of the disease) was 64% ($P < 0.01$). In the necrotizing AP group overall mortality rate was 25% ($P < 0.01$), and early mortality was 62% ($P < 0.01$), compared to the 1.8% mortality rate seen in the edematous pancreatitis group. The main differences in clinical course and outcome of patients with mild and severe disease are revealed in Table 1. Interestingly, both severe and necrotizing AP were associated with a significant reduction in number of circulating blood lymphocytes, although there was no statistically significant difference in total WBC count (Figure 2). The mean WBC count in the SAP group was $14.76 ± 0.95 × 10^9$/L compared to $13.92 ± 0.62 × 10^9$/L in the mild AP group (n.s.). The mean WBC count in the necrotizing AP group was $14.38 ± 0.91 × 10^9$/L compared to $14.27 ± 0.75 × 10^9$/L in the edematous AP group (n.s.). However, the number of circulating lymphocytes in peripheral blood was reduced by 25% in the SAP compared to the mild AP group ($P < 0.05$), and by 17% in the necrotizing AP group compared to the edematous AP group ($P < 0.05$). Based on the significance and clinical relevance of these findings we investigated the presence of possible common molecular pathways leading to development of severe and necrotizing AP.
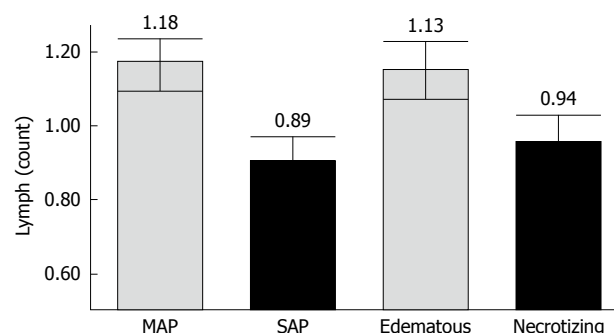
Figure 2 Reduction in number of lymphocytes during severe and necrotizing AP. Number of circulating lymphocytes in peripheral blood was significantly reduced in severe acute pancreatitis (SAP) compared to mild AP groups ($0.89 ± 0.08 × 10^9$/L *vs* $1.18 ± 0.07 × 10^9$/L, $P < 0.05$), and in necrotizing compared to edematous pancreatitis groups ($0.94 ± 0.08 × 10^9$/L *vs* $1.13 ± 0.08 × 10^9$/L, $P < 0.05$). These findings demonstrate the presence of possible common molecular pathways leading to the development of severe and necrotizing AP.

QRT-PCR analysis of cytokine mRNA expression levels in patients with severe and mild disease revealed some conflicting results (Figure 3A). Firstly, study showed that SAP is characterized by decreased intracellular mRNA levels of interleukin (IL)-1 and interferon-γ (IFN-γ). Secondly, SAP was also associated with a marked decrease in the levels of CD25 and CD69 mRNA in peripheral blood leukocytes. However, severe and necrotizing pancreatitis was associated with a very high activation of neutrophils and macrophages as demonstrated by nearly ten-fold higher mRNA expression of elastase and myeloperoxidase in peripheral WBCs. Another quite unexpected result showed a significantly lower mRNA expression of ICAM-1 and macrophage migration inhibitory factor (MIF) in peripheral WBCs (but not in the blood serum) from patients with SAP compared to that of mild AP patients. Analysis of cytokine protein expression levels showed that SAP was associated with a typical SIRS and 2-5 fold increase in expression of pro-inflammatory cytokines (IL-6, IL-8, MIF), as well as induction of compensatory and regulatory mechanism (IL-10) (Figure 3B). Enormously high levels of leukotriene B4 (LTB4) associated with neutrophil activation and injury to the vital organs were recorded in patients who died during the early stages of the disease.

Another important issue is whether the serum soluble molecules could be used as accurate early markers to predict the development of local (pancreatic necrosis) and systemic complications (SIRS, MOF) later in the course of the disease. Discrimination function analysis was used to determine differences between the groups of patients with mild and severe AP, edematous and necrotizing AP, and between survivors and those with fatal outcome. We included five variables (IL-6, IL-8, IL-10, MIF and LTB4) in a study in order to find out which serum markers are the best discriminators of the above mentioned groups. At each step, the variable that minimized the overall Wilks' Lambda was entered into the discriminant model thus revealing that IL-6 and MIF values were significant discriminators between groups of patients with mild and severe disease, as well as between survivors and non-survivors.

Table 2 Serum IL-6 and MIF are significant discriminators between groups of AP patients

Step	Entered	Wilks' Lambda							
		Statistic	df1	df2	df3	Exact F			
						Statistic	df1	df2	*P*
Severe *vs* mild									
1	IL-6	0.739	1	1	70.000	24.696	1	70.000	0.000
2	MIF	0.675	2	1	70.000	16.635	2	69.000	0.000
Edematous *vs* necrotizing									
1	IL-6	0.851	1	1	70.000	12.261	1	70.000	0.001
2	MIF[1]	0.943	1	1	70.000	4.247	1	70.000	0.043
Survivors *vs* non-survivors									
1	IL-6	0.761	1	1	70.000	22.005	1	70.000	0.000
2	MIF	0.709	2	1	70.000	14.153	2	69.000	0.000

[1]Not included in the original model using the standard stepwise method due to its low discrimination value. At each step, the variable that minimizes the overall Wilks' Lambda is entered. IL-6: Interleukin-6; MIF: Macrophage migration inhibitory factor.
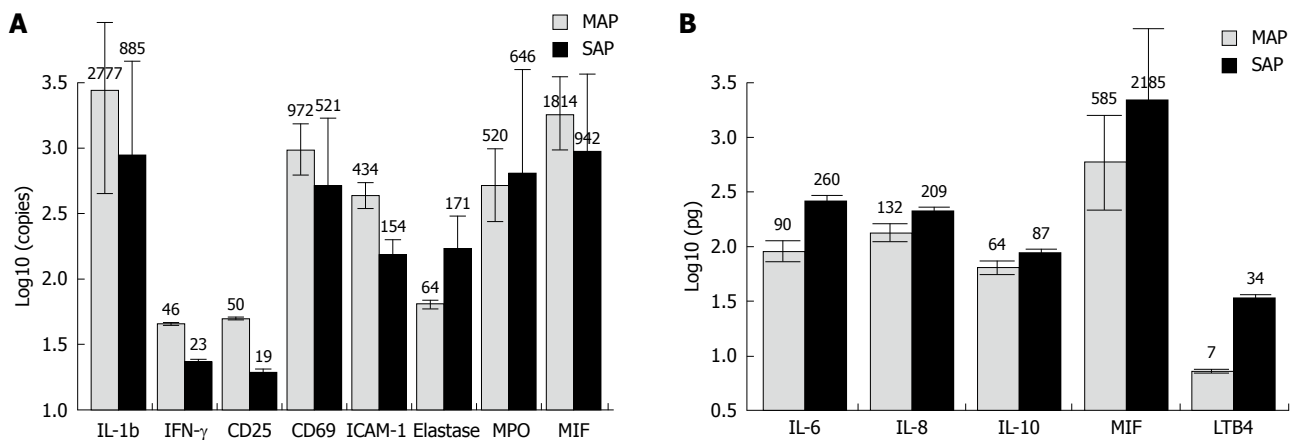
Figure 3 Cytokine expression at mRNA and protein level in peripheral blood of patients with mild and severe AP. A: Gene expression in peripheral blood leukocytes of MAP and SAP patients. QRT-PCR analysis revealed that SAP is characterized by significantly decreased intracellular mRNA levels of interleukin (IL)-1 and interferon (IFN)-γ. SAP is also associated with markedly lower mRNA expression levels of CD25 and CD69 in peripheral blood leukocytes. However, severe and necrotizing pancreatitis was associated with a very high activation of neutrophils and macrophages as demonstrated by nearly ten-fold higher mRNA expression of elastase and myeloperoxidase in peripheral white blood cells (WBCs). A significantly lower mRNA expression of ICAM-1 and macrophage migration inhibitory factor (MIF) was observed in peripheral WBCs (but not in the blood serum) from patients with SAP compared to that of MAP group. Cytokine mRNA expression levels with statistically significant differences between MAP and SAP groups are depicted; B: Cytokine concentration in blood serum of MAP and SAP patients. Analysis of cytokine expression at protein levels shows that SAP is associated with a typical systemic inflammatory response syndrome (SIRS) and 2-5 fold increase in expression of pro-inflammatory cytokines (IL-6, IL-8, MIF). Induction of a compensatory and regulatory mechanism (IL-10) is also observed in this group. Up to 4-5 fold higher levels of LTB4 were recorded in patients who died during the early stages of the disease. Cytokine expression levels with statistically significant differences between AP and healthy control groups are shown.
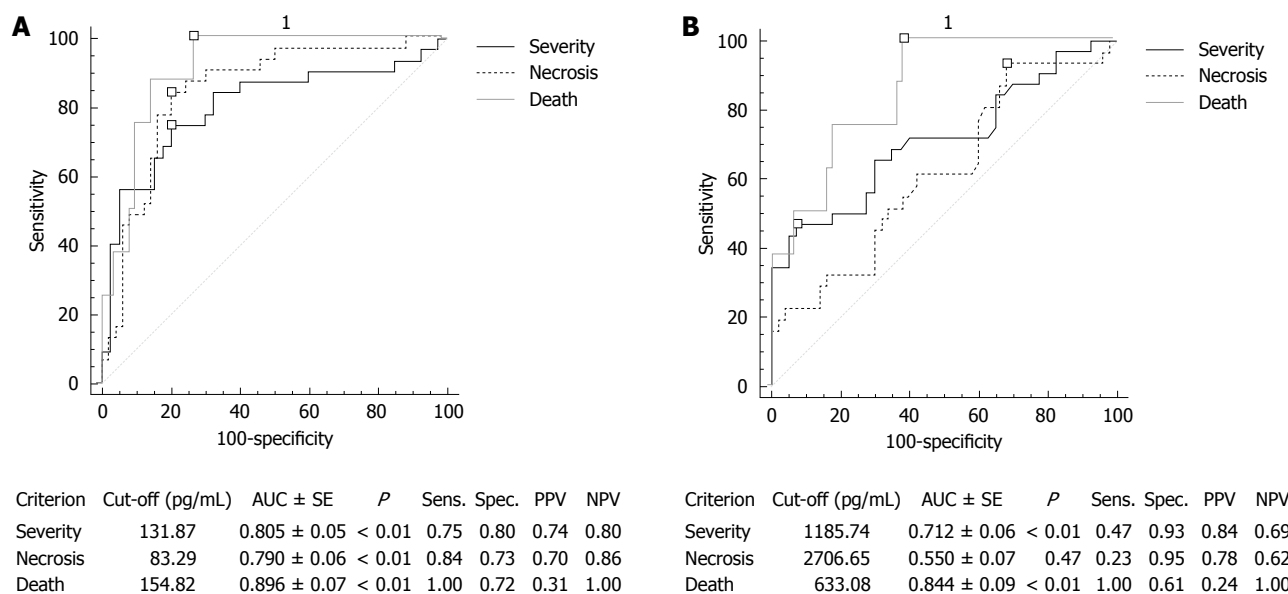
Statistical analysis revealed that IL-6 values differed significantly in groups of patients with and without pancreatic necrosis (Table 2). A particular cut-off value for IL-6 and MIF was established using Receiver Operating Characteristic (ROC) curve analysis to practically discriminate cases with severe, necrotizing and fatal acute pancreatitis (Figure 4A and B). Comparison of ROC curves for IL-6 and MIF showed no significant differences in diagnostic performance of these two routine tests, except for the discrimination of edematous and necrotizing pancreatitis where IL-6 proved to be of superior value.

DISCUSSION

Alteration of the immune system is one of the major mechanisms responsible for early and late mortality in severe AP. Excessive inflammatory reaction, known as systemic inflammatory response syndrome (SIRS), is considered as the leading cause of death in early AP[14,19,20,29]. The role of lymphocytes in this phenomenon has been partly studied and the knowledge of the mechanisms in humans is still incomplete[20,30-35]. In agreement with previous studies, we found a significant depletion of circulating lymphocytes which was much more profound in the severe and necrotizing forms of pancreatitis[20,30-35]. In our study, significant activation of lymphocytes was observed in the case of AP compared to healthy controls, as shown by strong expression of CD69 and CD25. The surface receptors CD69 and CD25 (IL-2 receptor) are early markers of lymphocyte activation. Increased expression of CD69 as well as of CD25 on CD3+, CD4+ and CD8+ cells have recently been reported in mild AP. It has also been suggested that the number of B-lymphocytes (CD19+) expressing CD69 was significantly lower in patients with

Criterion	Cut-off (pg/mL)	AUC ± SE	P	Sens.	Spec.	PPV	NPV
Severity	131.87	0.805 ± 0.05	< 0.01	0.75	0.80	0.74	0.80
Necrosis	83.29	0.790 ± 0.06	< 0.01	0.84	0.73	0.70	0.86
Death	154.82	0.896 ± 0.07	< 0.01	1.00	0.72	0.31	1.00

Criterion	Cut-off (pg/mL)	AUC ± SE	P	Sens.	Spec.	PPV	NPV
Severity	1185.74	0.712 ± 0.06	< 0.01	0.47	0.93	0.84	0.69
Necrosis	2706.65	0.550 ± 0.07	0.47	0.23	0.95	0.78	0.62
Death	633.08	0.844 ± 0.09	< 0.01	1.00	0.61	0.24	1.00

Figure 4 Prognostic utility of IL-6 and MIF in diagnosis of severe, necrotizing and fatal AP. A: Performance of serum IL-6 in diagnosis of severe, necrotizing and fatal AP. The diagnostic performance of a test for discriminating severe, necrotizing and fatal cases of AP was evaluated using Receiver Operating Characteristic (ROC) curve analysis. Study revealed that serum IL-6 concentration is a good predictor of the severe disease and systemic complications (SIRS, MOF); this marker could also be utilized for the stratification of patients with necrotizing AP and those with possible fatal outcome; B: Performance of serum MIF in diagnosis of severe, necrotizing and fatal AP. The diagnostic performance of a test for discriminating severe, necrotizing and fatal cases of AP was evaluated using ROC curve analysis. Study revealed that serum MIF concentration is a good predictor of the severe disease and systemic complications (SIRS, MOF); this marker could be used for early identification of patients with possible fatal outcome. Comparison of ROC curves for IL-6 and MIF showed no statistically significant differences in this respect (P = 0.18 for severity; P = 0.58 for mortality). However, serum MIF concentration has very poor prognostic value in predicting the development of pancreatic necrosis and is inferior to IL-6 serum concentration in this respect (P < 0.01). AUC: Area under curve; Sens.: Sensitivity; Spec.: Specificity; PPV: Positive predictive value; NPV: Negative predictive value.

severe pancreatitis than in patients with mild pancreatitis[20,31,32]. We have also observed a significantly lower expression of both CD69 and CD25 surface receptors in peripheral blood leukocytes from SAP patients. Marked activation of different subsets of lymphocytes may explain why we have observed strong elevation of different cytokines in peripheral blood, despite a significant decrease in the number of circulating lymphocytes. One of the possible reasons for the decreased number of circulating lymphocytes is strong migration of activated cells to the sites of inflammation, including the pancreas and other tissues such as lungs or kidneys, as an element of SIRS[16,20,36]. Another possible explanation is an excessive elimination of lymphocytes by apoptosis. Interestingly, the available data indicate that lymphopenia caused by the decrease of different lymphocyte subpopulations is also characteristic for other diseases with severe tissue damage, e.g. critical injury, sepsis and acute severe respiratory syndrome (ARDS)[37-40].

Polymorphonuclear leukocytes, monocytes, tissue macrophages, lymphocytes, natural killer cells, and parenchymal cells are involved in a complex network of the host defense response in the case of tissue damage caused by severe AP, burns, accident trauma, major surgical interventions or sepsis[20,39,41,42]. An overwhelming pro-inflammatory response (hyperinflammation) leads to the clinical manifestations of SIRS and finally to host defence failure expressed by multiple organ failure (MOF). The up-regulation of pro-inflammatory factors, such as TNF, IL-1 and IL-6, observed during the SIRS phase can be followed by

a second response that involves down-regulation of IFN-γ and increase in anti-inflammatory cytokines, such as IL-10 and transforming growth factor-β. This counter regulatory phenomenon is called the compensatory anti-inflammatory response syndrome (CARS)[17,20,39]. However, this can result in the development of immunosuppression and is often associated with elevation of IL-10 and the shift of the Th1/Th2 balance towards a Th2 response[17,20,39,42]. The network of various cytokines and other molecules participating in the regulation of the inflammatory processes is indeed very complex, and the precise timing of the release and activation of these mediators is not well known. Nevertheless, our study also shows that both SIRS and CARS occur in a real clinical situation and at a certain stage of the disease might even develop simultaneously. However, in the case of severe AP, the counter-regulatory mechanisms may lead to the dysfunction of immune cells mainly due to the significant decrease in total systemic lymphocyte count and the hyper-production of IL-10, which might suggest that T regulatory 1 cells (Tr1) are especially active in the course of severe disease[43].

Involvement of other cellular subsets of leukocytes, mainly neutrophils and monocyte-macrophage lineage, in the development of SAP is also readily supported by our study. Neutrophils exert their toxicity by releasing myeloperoxidase (MPO), proteases such as elastase and reactive oxygen species (ROS) such as H_2O_2[36]. In our study we have observed a very high mRNA expression of MPO and polymorphonuclear leukocyte elastase in cases of severe and necrotizing pancreatitis. Increased production

of platelet activating factor (PAF) and LTB4 is also closely associated with an activation of monocytes and neutrophils, and increased migration of neutrophils to pancreas and other target organs, including lungs and kidneys[36,44,45]. We have also recorded noticeably higher levels of serum LTB4 in patients suffering from severe and fatal acute pancreatitis. Furthermore, patients with severe and necrotizing AP exerted very high serum levels of MIF. MIF is predominantly released by monocytes, macrophages and Th2 T-cell clones; it is associated with activation of immune cells and release of pro-inflammatory cytokines overriding glucocorticoid action at the site of inflammation. It is also known to be associated with a lung injury during SIRS through counter-regulation of the effect of glucocorticoids and through up-regulation of macrophage inflammatory protein-2, which itself serves as a potent chemo-attractant to the neutrophils[46-49].

The infiltration of inflammatory cells into the pancreas is an early and central event in acute pancreatitis that promotes local injury and systemic complications of the disease. Recent research has yielded the important finding that resident cells of the pancreas (particularly acinar and pancreatic stellate cells) play an important role in leukocyte attraction *via* secretion of chemokines, cytokines and expression of adhesion molecules[36,50-53]. We believe this could at least partially explain the occurrence of systemic lymphopenia, lower CD25, CD69 and ICAM-1 mRNA expression in cases of SAP. Activation of inflammatory cells and release of cytokines into the systemic bloodstream at the site of injury is possibly also an explanation for the discrepancy of low MIF mRNA expression in peripheral WBCs in association with high serum concentration of MIF in the case of severe and necrotizing pancreatitis.

Having identified the inflammatory mediators that are significantly up-regulated in serum of patients with severe and necrotizing AP (IL-6, IL-8, IL-10, MIF and LTB4) in comparison to those with mild disease, we have also evaluated the prognostic utility of these molecules in predicting the negative outcome of the disease. In accordance with the findings of other studies we have confirmed that both serum IL-6 and MIF concentrations are very good discriminators of severe AP accompanied by MOF, and could be used as early markers of possible fatal outcome. However, in contrast to the recent study of Rahman *et al*[54], we failed to demonstrate that MIF is an early specific prognostic marker for the development of pancreatic necrosis. In our experience, serum IL-6 concentration was superior in this respect[28,55-57]. Nonetheless, serum MIF concentration may serve as an important prognostic marker that could identify patients likely to benefit from aggressive medical treatment and early transfer to specialist pancreatic units. At the molecular level, targeted disruption of these proteins with monoclonal antibodies may afford some therapeutic benefit in preventing the development of MOF, pancreatic necrosis, and septic complications.

Deregulation of the cellular immune system is a key event leading to the development of severe and necrotizing AP. Severe and necrotizing AP is characterized by the significant depletion of circulating lymphocytes in the bloodstream as a result of increased migration of these cells to the site of injury and other vital organs, including lungs and kidneys. Infiltration of pancreas and other peripheral organs by aberrantly activated inflammatory cells occurs within the first hours after onset of acute pancreatitis and leads to the development of SIRS and MOF. This whole process is regulated by a large number of cytokines and is an extremely complex network of intercellular interaction. It is clear that detailed elucidation of the numerous processes in the inflammatory cascade is an essential step towards the development of improved therapeutic strategies in acute pancreatitis. Published studies suggest that combination therapy targeting different steps of the inflammatory cascade may be the treatment of choice for this disease.

Data from other authors and from our current study demonstrate that serum IL-6 and MIF concentrations are very good discriminators of severe and necrotizing AP as well as of possible fatal outcome during the first hours and days after the onset of the disease. Therefore, these molecules could be used as markers for patient stratification and/or predicting the outcomes of AP in the early stages of the disease. However, their true prognostic utility, clinical and health care resource implications need to be further evaluated in a larger patient population and in the context of other currently existing diagnostic markers and indices.

COMMENTS

Background
Mortality of patients with severe acute pancreatitis (SAP) approaches 30%-40%. An imbalance between the early systemic inflammatory response syndrome (SIRS), and the later compensatory counter-inflammatory response, and development of multiple organ failure (MOF) are considered to be the primary causes of morbidity and mortality in SAP. Excessive leukocyte activation (including neutrophils and monocyte-macrophage lineage) with cytokinemia play a critical role in the pathogenesis of pancreatitis and even more so, of the subsequent inflammatory response. Thus, the aims of this study have been to investigate the secretion patterns of pro- and anti-inflammatory cytokines, and to estimate the activation of various cellular subsets of leukocytes (including lymphocytes, neutrophils, and monocyte-macrophage lineage) in peripheral blood of patients with severe and mild AP.

Research frontiers
It has been hypothesized that fatal pancreatitis is a consequence of abnormal phagocytic leukocyte hyperstimulation due to deregulation in T- and B-lymphocyte activation. However, the role of lymphocyte activation and its relation to the severity of disease in humans is still poorly understood. Another important drawback is that the majority of information about the alterations of the immune system during the AP comes from *in vitro* and *in vivo* studies, and therefore is not always directly applicable and relevant to the clinical situation in human AP.

Innovations and breakthroughs
This study by Dambrauskas *et al* confirms that human severe and necrotizing AP is characterized by the significant depletion of circulating lymphocytes. SAP is associated with a severe systemic inflammatory response syndrome (SIRS) and over-expression of pro-inflammatory cytokines [interleukin (IL)-6, IL-8, macrophage migration inhibitory factor (MIF)]. The study demonstrates that serum IL-6 and MIF concentrations are the best discriminators of severe and necrotizing AP as well as possible fatal outcome during the early course of the disease. These cytokines could also be used as early predictors of local (pancreatic necrosis) and systemic complications (SIRS, MOF) later in the course of the disease. Deregulation of the cellular immune system is a key event leading to

the development of severe and necrotizing AP. Infiltration of peripheral organs by aberrantly activated inflammatory cells leads to the development of MOF.

Applications
Data from other authors and from the authors' current study demonstrate that serum IL-6 and MIF concentrations are very good discriminators of severe and necrotizing AP as well as possible fatal outcome during the first hours and days after the onset of the disease. Therefore, these molecules could be used as markers for patient stratification and/or predicting the outcome of AP in the early stages of the disease.

Terminology
Pancreatitis is a condition associated with development of acute and sudden inflammation of the pancreas. In about 85% of patients, acute pancreatitis is a mild disease and is usually associated with a rapid recovery within a few days of onset of the illness. However, in 15%-20% of patients with acute pancreatitis, severe damage to the pancreas may lead to a life threatening illness that is often associated with prolonged hospitalization, multiple surgical procedures, development of multiple organ failure, severe infective complications, and death in some patients.

Peer review
This prospective observational study seeks to characterise the cytokine expression profiles (at mRNA and protein levels) of patients with acute pancreatitis and to correlate the profiles with disease severity.

REFERENCES

1   **Yousaf M**, McCallion K, Diamond T. Management of severe acute pancreatitis. *Br J Surg* 2003; **90**: 407-420

2   **Karimgani I**, Porter KA, Langevin RE, Banks PA. Prognostic factors in sterile pancreatic necrosis. *Gastroenterology* 1992; **103**: 1636-1640

3   **Toouli J**, Brooke-Smith M, Bassi C, Carr-Locke D, Telford J, Freeny P, Imrie C, Tandon R. Guidelines for the management of acute pancreatitis. *J Gastroenterol Hepatol* 2002; **17** Suppl: S15-S39

4   **Abu-Zidan FM**, Bonham MJ, Windsor JA. Severity of acute pancreatitis: a multivariate analysis of oxidative stress markers and modified Glasgow criteria. *Br J Surg* 2000; **87**: 1019-1023

5   **Isaji S**, Takada T, Kawarada Y, Hirata K, Mayumi T, Yoshida M, Sekimoto M, Hirota M, Kimura Y, Takeda K, Koizumi M, Otsuki M, Matsuno S. JPN Guidelines for the management of acute pancreatitis: surgical management. *J Hepatobiliary Pancreat Surg* 2006; **13**: 48-55

6   **Triester SL**, Kowdley KV. Prognostic factors in acute pancreatitis. *J Clin Gastroenterol* 2002; **34**: 167-176

7   **Balthazar EJ**, Freeny PC, vanSonnenberg E. Imaging and intervention in acute pancreatitis. *Radiology* 1994; **193**: 297-306

8   **Bradley EL 3rd**. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. *Arch Surg* 1993; **128**: 586-590

9   **Isenmann R**, Rau B, Beger HG. Bacterial infection and extent of necrosis are determinants of organ failure in patients with acute necrotizing pancreatitis. *Br J Surg* 1999; **86**: 1020-1024

10 **Bradley EL 3rd**, Allen K. A prospective longitudinal study of observation versus surgical intervention in the management of necrotizing pancreatitis. *Am J Surg* 1991; **161**: 19-24; discussion 24-25

11 **Gloor B**, Müller CA, Worni M, Martignoni ME, Uhl W, Büchler MW. Late mortality in patients with severe acute pancreatitis. *Br J Surg* 2001; **88**: 975-979

12 **Rattner DW**, Legermate DA, Lee MJ, Mueller PR, Warshaw AL. Early surgical débridement of symptomatic pancreatic necrosis is beneficial irrespective of infection. *Am J Surg* 1992; **163**: 105-109; discussion 109-110

13 **Uhl W**, Warshaw A, Imrie C, Bassi C, McKay CJ, Lankisch PG, Carter R, Di Magno E, Banks PA, Whitcomb DC, Dervenis C, Ulrich CD, Satake K, Ghaneh P, Hartwig W, Werner J, McEntee G, Neoptolemos JP, Büchler MW. IAP Guidelines for the Surgical Management of Acute Pancreatitis. *Pancreatology* 2002; **2**: 565-573

14 **Bhatia M**, Brady M, Shokuhi S, Christmas S, Neoptolemos JP, Slavin J. Inflammatory mediators in acute pancreatitis. *J Pathol* 2000; **190**: 117-125

15 **Bhatia M**, Neoptolemos JP, Slavin J. Inflammatory mediators as therapeutic targets in acute pancreatitis. *Curr Opin Investig Drugs* 2001; **2**: 496-501

16 **Bhatia M**, Wong FL, Cao Y, Lau HY, Huang J, Puneet P, Chevali L. Pathophysiology of acute pancreatitis. *Pancreatology* 2005; **5**: 132-144

17 **Ni Choileain N**, Redmond HP. The immunological consequences of injury. *Surgeon* 2006; **4**: 23-31

18 **Kusske AM**, Rongione AJ, Reber HA. Cytokines and acute pancreatitis. *Gastroenterology* 1996; **110**: 639-642

19 **Norman J**. The role of cytokines in the pathogenesis of acute pancreatitis. *Am J Surg* 1998; **175**: 76-83

20 **Pietruczuk M**, Dabrowska MI, Wereszczynska-Siemiatkowska U, Dabrowski A. Alteration of peripheral blood lymphocyte subsets in acute pancreatitis. *World J Gastroenterol* 2006; **12**: 5344-5351

21 **Ueda T**, Takeyama Y, Yasuda T, Shinzeki M, Sawa H, Nakajima T, Ajiki T, Fujino Y, Suzuki Y, Kuroda Y. Immunosuppression in patients with severe acute pancreatitis. *J Gastroenterol* 2006; **41**: 779-784

22 **Knaus WA**, Draper EA, Wagner DP, Zimmerman JE. Prognosis in acute organ-system failure. *Ann Surg* 1985; **202**: 685-693

23 **Pezzilli R**, Billi P, Miniero R, Fiocchi M, Cappelletti O, Morselli-Labate AM, Barakat B, Sprovieri G, Miglioli M. Serum interleukin-6, interleukin-8, and beta 2-microglobulin in early assessment of severity of acute pancreatitis. Comparison with serum C-reactive protein. *Dig Dis Sci* 1995; **40**: 2341-2348

24 **Ranson JH**. Etiological and prognostic factors in human acute pancreatitis: a review. *Am J Gastroenterol* 1982; **77**: 633-638

25 **Ranson JH**, Balthazar E, Caccavale R, Cooper M. Computed tomography and the prediction of pancreatic abscess in acute pancreatitis. *Ann Surg* 1985; **201**: 656-665

26 **Wilson C**, Heads A, Shenkin A, Imrie CW. C-reactive protein, antiproteases and complement factors as objective markers of severity in acute pancreatitis. *Br J Surg* 1989; **76**: 177-181

27 **Wilson C**, Heath DI, Imrie CW. Prediction of outcome in acute pancreatitis: a comparative study of APACHE II, clinical assessment and multiple factor scoring systems. *Br J Surg* 1990; **77**: 1260-1264

28 **Dambrauskas Z**, Pundzius J, Barauskas G. Predicting development of infected necrosis in acute necrotizing pancreatitis. *Medicina* (Kaunas) 2006; **42**: 441-449

29 **Ogawa M**. Acute pancreatitis and cytokines: "second attack" by septic complication leads to organ failure. *Pancreas* 1998; **16**: 312-315

30 **Curley PJ**, McMahon MJ, Lancaster F, Banks RE, Barclay GR, Shefta J, Boylston AW, Whicher JT. Reduction in circulating levels of CD4-positive lymphocytes in acute pancreatitis: relationship to endotoxin, interleukin 6 and disease severity. *Br J Surg* 1993; **80**: 1312-1315

31 **Pezzilli R**, Billi P, Beltrandi E, Maldini M, Mancini R, Morselli Labate AM, Miglioli M. Circulating lymphocyte subsets in human acute pancreatitis. *Pancreas* 1995; **11**: 95-100

32 **Pezzilli R**, Maldini M, Morselli-Labate AM, Barakat B, Romboli E, Beltrandi E, Migliori M, Tomassetti P, Corinaldesi R. Early activation of peripheral lymphocytes in human acute pancreatitis. *J Clin Gastroenterol* 2003; **36**: 360-363

33 **Sweeney KJ**, Kell MR, Coates C, Murphy T, Reynolds JV. Serum antigen(s) drive the proinflammatory T cell response in acute pancreatitis. *Br J Surg* 2003; **90**: 313-319

34 **Takeyama Y**, Takas K, Ueda T, Hori Y, Goshima M, Kuroda Y. Peripheral lymphocyte reduction in severe acute pancre-

atitis is caused by apoptotic cell death. *J Gastrointest Surg* 2000; **4**: 379-387

35 **Widdison AL**, Cunningham S. Immune function early in acute pancreatitis. *Br J Surg* 1996; **83**: 633-636

36 **Vonlaufen A**, Apte MV, Imhof BA, Frossard JL. The role of inflammatory and parenchymal cells in acute pancreatitis. *J Pathol* 2007; **213**: 239-248

37 **Chen CY**, Lee CH, Liu CY, Wang JH, Wang LM, Perng RP. Clinical features and outcomes of severe acute respiratory syndrome and predictive factors for acute respiratory distress syndrome. *J Chin Med Assoc* 2005; **68**: 4-10

38 **Fernández-Fresnedo G**, Ramos MA, González-Pardo MC, de Francisco AL, López-Hoyos M, Arias M. B lymphopenia in uremia is related to an accelerated in vitro apoptosis and dysregulation of Bcl-2. *Nephrol Dial Transplant* 2000; **15**: 502-510

39 **Smith JW**, Gamelli RL, Jones SB, Shankar R. Immunologic responses to critical injury and sepsis. *J Intensive Care Med* 2006; **21**: 160-172

40 **Sun Q**, Liu ZH, Chen J, Ji S, Tang Z, Cheng Z, Ji D, Li LS. An aggressive systematic strategy for acute respiratory distress syndrome caused by severe pneumonia after renal transplantation. *Transpl Int* 2006; **19**: 110-116

41 **Keel M**, Trentz O. Pathophysiology of polytrauma. *Injury* 2005; **36**: 691-709

42 **Menger MD**, Vollmar B. Surgical trauma: hyperinflammation versus immunosuppression? *Langenbecks Arch Surg* 2004; **389**: 475-484

43 **Allan SE**, Broady R, Gregori S, Himmel ME, Locke N, Roncarolo MG, Bacchetta R, Levings MK. CD4+ T-regulatory cells: toward therapy for human diseases. *Immunol Rev* 2008; **223**: 391-421

44 **Hotter G**, Closa D, Prats N, Pi F, Gelpí E, Roselló-Catafau J. Free radical enhancement promotes leucocyte recruitment through a PAF and LTB4 dependent mechanism. *Free Radic Biol Med* 1997; **22**: 947-954

45 **Pastor CM**, Vonlaufen A, Georgi F, Hadengue A, Morel P, Frossard JL. Neutrophil depletion--but not prevention of Kupffer cell activation--decreases the severity of cerulein-induced acute pancreatitis. *World J Gastroenterol* 2006; **12**: 1219-1224

46 **Bacher M**, Metz CN, Calandra T, Mayer K, Chesney J, Lo-hoff M, Gemsa D, Donnelly T, Bucala R. An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. *Proc Natl Acad Sci USA* 1996; **93**: 7849-7854

47 **Baugh JA**, Bucala R. Macrophage migration inhibitory factor. *Crit Care Med* 2002; **30**: S27-S35

48 **Calandra T**, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T, Cerami A, Bucala R. MIF as a glucocorticoid-induced modulator of cytokine production. *Nature* 1995; **377**: 68-71

49 **Calandra T**, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol* 2003; **3**: 791-800

50 **Gukovskaya AS**, Gukovsky I, Zaninovic V, Song M, Sandoval D, Gukovsky S, Pandol SJ. Pancreatic acinar cells produce, release, and respond to tumor necrosis factor-alpha. Role in regulating cell death and pancreatitis. *J Clin Invest* 1997; **100**: 1853-1862

51 **Mews P**, Phillips P, Fahmy R, Korsten M, Pirola R, Wilson J, Apte M. Pancreatic stellate cells respond to inflammatory cytokines: potential role in chronic pancreatitis. *Gut* 2002; **50**: 535-541

52 **Ramudo L**, Manso MA, Vicente S, De Dios I. Pro- and anti-inflammatory response of acinar cells during acute pancreatitis. Effect of N-acetyl cysteine. *Cytokine* 2005; **32**: 125-131

53 **Shimizu K**, Kobayashi M, Tahara J, Shiratori K. Cytokines and peroxisome proliferator-activated receptor gamma ligand regulate phagocytosis by pancreatic stellate cells. *Gastroenterology* 2005; **128**: 2105-2118

54 **Rahman SH**, Menon KV, Holmfield JH, McMahon MJ, Guillou JP. Serum macrophage migration inhibitory factor is an early marker of pancreatic necrosis in acute pancreatitis. *Ann Surg* 2007; **245**: 282-289

55 **Mayer J**, Rau B, Gansauge F, Beger HG. Inflammatory mediators in human acute pancreatitis: clinical and pathophysiological implications. *Gut* 2000; **47**: 546-552

56 **Dervenis CG**. Staging acute pancreatitis: where are we now? *Pancreatology* 2001; **1**: 201-206

57 **Sakai Y**, Masamune A, Satoh A, Nishihira J, Yamagiwa T, Shimosegawa T. Macrophage migration inhibitory factor is a critical mediator of severe acute pancreatitis. *Gastroenterology* 2003; **124**: 725-736

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