Anti-cytokine strategies in acute pancreatitis: pathophysiological insights and clinical implications

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Abstract

The clinical presentation of acute pancreatitis varies significantly from mild self-limiting discomfort to a severe life-threatening condition. Once the disease process is initiated, the severity of the disease is largely determined by a complex network of activated inflammatory mediators such as cytokines, proteolytic enzymes, reactive oxygen species, and many more which render the local injury to a systemic disease with multiple organ dysfunction, sepsis, and considerable mortality. Remarkable progress in diagnostic modalities, intensive care technologies, and organ preserving surgical techniques have decreased mortality of severe acute pancreatitis during the past decades. However, the treatment of acute pancreatitis still remains largely supportive and no specific approach exists to prevent evolving complications. A large body of clinical and experimental evidence suggests that cytokines are key factors in the pathomechanism of local and systemic complications of acute pancreatitis. Targeting cytokine activity as therapeutic approach to acute pancreatitis is a challenging concept and the results of modulating activation of TNF-α, IL-1β, IL-2, IL-10, PAF and various chemokines has indeed been promising in the experimental setting even if tested under therapeutic conditions. However, experience from a limited number of clinical trials on anti cytokine strategies in acute pancreatitis has remarkably emphasized that translating successful experimental observations into reproducible clinical associations seems to be difficult.

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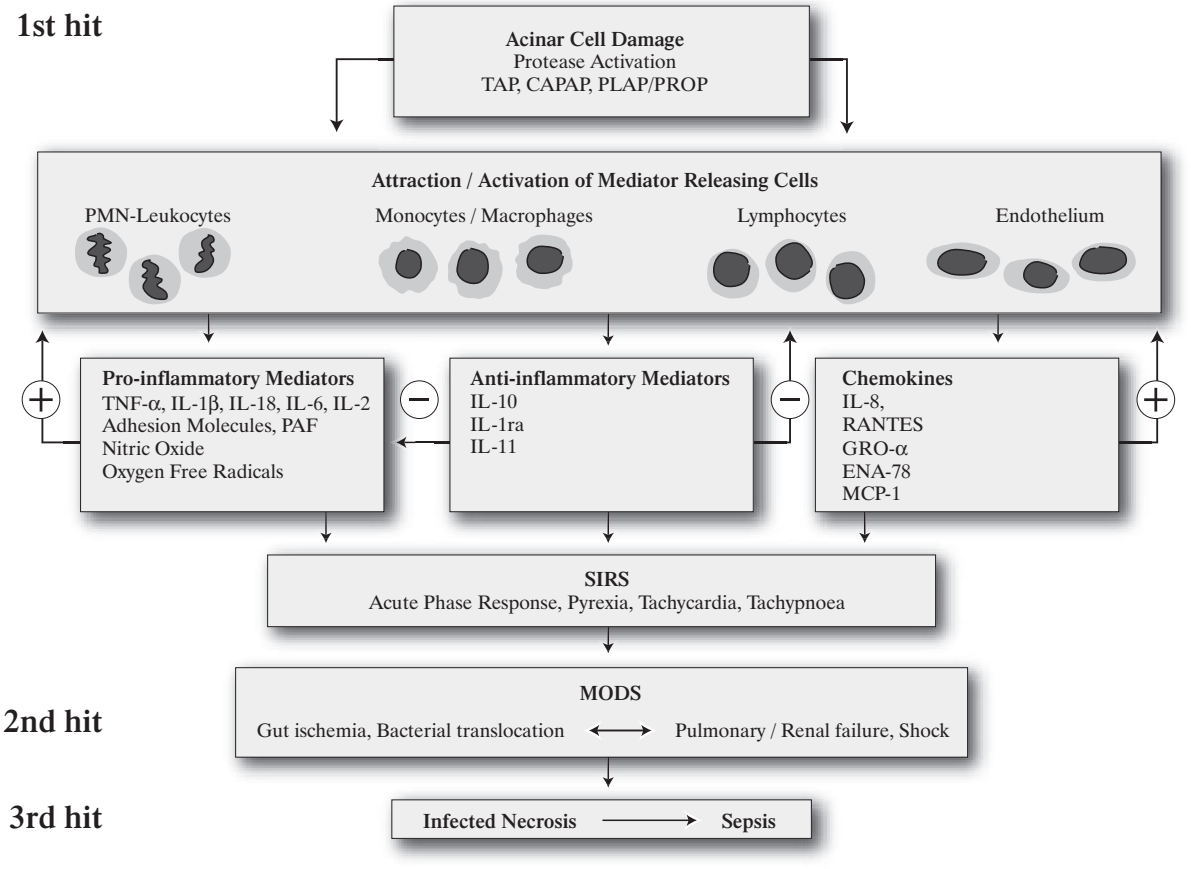
Introduction

Acute pancreatitis usually takes an uneventful course with complete restitutio ad intergrum and mortality rates of less than 1%. In contrast, in about 25% of all patients the disease takes a severe course evolving to a potentially life-threatening condition with considerable morbidity and mortality [1]. It has been largely shown that the hallmark of severe acute pancreatitis is the development of pancreatic necrosis which is still the essential determinant of further complications [2,3]. Depending on the extent of intra- and extrapancreatic necrosis pancreatic infections and remote organ failure frequently arise as major complications in the subsequent course of the disease and considerably add to mortality [2-6]. Nowadays, a multidisciplinary approach of prolonged intensive care management and delayed organ sparing surgical protocols has decreased the mortality of severe acute pancreatitis to about 20% to 30% in the past 20 years [7,8]. Despite the introduction of novel therapeutic concepts such as early ERCP in biliary acute pancreatitis, prophylactic antibiotics, and enteral nutrition since the early 90ies [7] the management of acute pancreatitis still remains largely supportive and despite all efforts, a break-trough in lowering mortality in patients with severe attacks has not been achieved [3-6]. Currently, no disease-specific medical treatment has ever been proven to overcome relevant complications such as pancreatic necrosis, infection of necrosis or organ failure effectively.

Dissatisfaction with persistently high mortality rates along with an improved understanding of the underlying pathomechanism of acute pancreatitis have made pancreatologists to pursue the search for alternative therapeutic approaches. More than 100 years ago Chiari proposed the first pathophysiological concept of acute pancreatitis by “autodigestion” of the gland via its own enzymes [9]. Since the mid 80ies, the pancreatic proteinase-antiproteinase imbalance was thought

Figure 1. **Schematic overview of the inflammatory cascade in acute pancreatitis. Activation of various leukocyte subsets and endothelium at the site of injury release various pro- and antiinflammatory cytokines, chemokines, and other mediators. An overt and sustained activation of proinflammatory mediators leads to systemic inflammatory response syndrome (SIRS) which may further proceed to multi organ dysfunction syndrome (MODS), infection of necrosis and sepsis**



to play a key role in the pathogenesis of acute pancreatitis. Hence, trypsinogen activation is believed to be one of the earliest pathophysiological events which triggers a cascade of other pancreatic proenzymes such as chymotrypsinogen, type I prophospholipase A_2, procarboxypeptidase B, or proelastase [10]. According to the "autodigestion" theory by Chiari, premature trypsinogen activation within the acinar cells has been found in various experimental models of acute pancreatitis [10,11]. Subsequently, significant amounts of trypsinogen and other proteases have been measured in the interstitial space as well as in the systemic circulation with a positive correlation to the extent of pancreatic tissue destruction and overall disease severity [11]. However, trypsinogen activation is only a temporary event in acute pancreatitis and most recent experimental studies have questioned the prevailing opinion of its dominating pathophysiological role [12]. These recent findings would at least in part explain the failure of antiproteinase therapy [13] or inhibiting pancreatic enzyme secretion [14] in decreasing complications and associated mortality in human acute pancreatitis.

An alternative pathophysiological concept was proposed by Rinderknecht in 1988. According to his theory, a complex network of inflammatory mediators released by activated leukocytes was suggested as key factor for rendering the local pancreatic insult into a systemic disease with distant organ failure [15]. In the subsequent years a growing number of clinical studies convincingly showed that acute pancreatitis is reflected by a large array of circulating inflammatory variables such as cytokines, chemokines, reactive oxygen species, adhesion molecules, acute phase proteins, and others [16,17]. In patients with an overt systemic inflammatory response and subsequent organ failure the quantitative release of nearly all mediators measured was significantly higher than that observed in patients with mild disease [18,19]. As a clinical consequence, measurement of specific inflammatory mediators offered a new interesting alternative to an easier severity stratification of acute pancreatitis compared with expensive imaging procedures or clinical staging scores [20]. Beyond the diagnostic and prognostic implications the "mediator hypothesis" was further substantiated by a growing number of experimental studies which ultimately lead to the establishment of the so-called "three" hit theory (*Fig. 1*). Herein, various pro- and antiinflammatory mediators are considered as important link between the initial local insult (first hit), the systemic host response and organ failure (second hit), and subsequent septic complications (third hit) in acute pancreatitis. By either inhibiting leukocyte activation or directly targeting leukocyte derived inflammatory mediators cytokines

Table 1. **Therapeutic effects of cytokine modulating approaches in experimental acute pancreatitis**

Author	Target	Model	Delay of drug administration	Intrapancreatic damage	Distat organ damage	Mortality
Norman et al. [35]	TNF-α	CDE, mouse	1.5 days	edema ↓	no effect	decrease
Norman et al. [43]	IL-1β	Cerulein, mouse	1 hour	necrosis, edema ↓	ND	ND
Norman et al. [45]	IL-1β	CDE, mouse	1.5 days	necrosis, edema ↓	lung ↓	decrease
Paszkowski et al. [48]	IL-1β/ICE	TC, rat	12 hours	necrosis ↓	lung ↓	decrease
Kusske et al. [75]	IL-10	CDE, mouse	33 hours	histologic score ↓	ND	decrease
Rongione et al. [76]	IL-10	Cerulein, rat	2 hours	histologic score ↓	ND	ND
Zou et al. [80]	IL-10	TC, rat	30 minutes	necrosis ↓	lung ↓, liver ↓	decrease
Mayer et al. [89]	IL-2	Cerulein, mouse	6 hours	histologic score ↓	lung ↓	ND
Formela et al. [99]	PAF	Ischemia, rat	30 minutes	histologic score ↓	ND	ND
Hofbauer et al. [101]	PAF	Duct ligation, opossum	2 days	necrosis ↓	lung ↓	ND
Foitzik et al. [102]	PAF	GDOC, rat	6 hours	microcirculation ↓	lung, kidney function ↓	decrease
Bhatia et al. [117]	CINC	Cerulein, rat	1 hour	no effect	lung ↓	ND
Bhatia et al. [118]	RANTES	Cerulein, mouse	1 hour	no effect	lung ↓	ND
Bhatia et al. [120]	MCP-1	Cerulein, mouse	1 hour	necrosis ↓	ND	ND

CDE – choline deficient ethionine supplemented; TC – Taurocholate; GDOC – Glycodeoxycholic acid; ND – not determined

have been recognized as central determinants of severity in acute pancreatitis and emerged as interesting targets for a potential therapeutic approach (*Tab. 1*).

The Role of Cytokines

Cytokines are a family of low molecular weight proteins (16-28 kDa), which have been extensively investigated in inflammatory conditions including acute pancreatitis. More than 30 different cytokines have been identified so far. With few exceptions, cytokines are not constitutively expressed in normal tissues and upregulation is usually initiated following external stimuli such as injury or stress in various cell types [16,17]. All cytokines cause their effects via highly specific membrane bound cell-surface receptors and have pleiotropic activities on a variety of target cells. On a functional basis cytokines can be divided into two groups: the pro- and the anti-inflammatory cytokines. There is immense redundancy within the system in such that many cytokines share similar biological effects and in the absence of another, they can fill the gap. Currently, there is no more doubt about the detrimental role of many cytokines in promoting local tissue destruction and mediating distant organ complications in acute pancreatitis and inflammatory disorders in general.

Tumor necrosis factor-α (TNF-α) and Interleukin-1β

Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) belong to the so-called "first order" proinflammatory cytokines. Activated macrophages and polymorphonuclear leukocytes have been thought to be the primary site and major source of TNF-α and IL-1β synthesis for years [18,21]. However, more recent studies have emphasized the impact of acinar cells in contributing to the synthesis and release of TNF-α and other cytokines [22-25]. A primary involvement of TNF-α in acute pancreatitis was shown in both clinical [26] and experimental studies [27-30] already since the early 90ies. Besides an early rise of systemic TNF-α concentrations [27-29] organ-specific and time dependent upregulation of TNF-α mRNA and protein levels in the pancreas but also in distant organs such as lung and liver provided the first evidence that cytokines are important mediators of systemic complications and survival [29-31]. In the experimental setting TNF-α antagonism by either anti TNF-α antibodies or TNF-α receptor blockade almost uniformly revealed protective effects on local intrapancreatic damage, systemic severity, and mortality [32-36]. An effective amelioration of pancreatitis associated pulmonary damage could be shown by alternative anti TNF strategies using inhibitors of p38 mitogen-activated protein (MAP) kinases of nuclear factor κB (NFκB) [23,37,38]. Interestingly, the protective effect of TNF-α antagonism on disease severity and mortality was still observed in a therapeutic study design after the systemic effects already had fully developed [35].

Similar observations have been made for IL-1β, the second of the "first order" cytokines. As observed for TNF-α, organ-specific expression of IL-1β is an early feature in experimental acute pancreatitis and is found in both the pancreas and distant organs [21,31] and correlates with the severity of the model studied. However, in contrast to an overt local overexpression, systemic IL-1β concentrations remain relatively low [19,39]. Unlike TNF-α, IL-1β synthesis and release has been mainly demonstrated by activated leukocyte populations [21,18] and no direct effect of this cytokine on acinar cell viability or function has ever been demonstrated [40,41]. Blockade of the IL-1 receptor by pharmacological agents or targeted genetic disruption revealed a significant reduction of intrapancreatic damage, systemic severity, and mortality in every established pancreatitis model similar to that observed by blocking TNF-α [36,42-45]. A recent interesting alternative approach to inhibit IL-1β activation in acute pancreatitis included the inhibition of caspase-1, formerly termed interleukin 1β-converting enzyme

(ICE). Targeting ICE activity by a specific synthetic inhibitor led to a dramatic amelioration of severity and mortality irrespective of the model used [46-48]. Of specific interest is the fact that the protective effects on overall severity and mortality were still present after a therapeutic window of 12 hours following induction of severe acute pancreatitis [48]. By comparing anti TNF-α and anti IL-1β strategies, it becomes clearly evident that both cytokines share striking similarities in their pathophysiological functions and closely control the regulation of their own and each other [44,49]. This was convincingly shown by Denham et al. [36] who could not demonstrate any additive protective effects by combined genetic disruption of TNF-α and the IL-1 receptor in two models of murine acute pancreatitis.

Surprisingly, in contrast to their outstanding pathophysiological importance both cytokines play no role as biochemical markers for a reliable severity assessment of acute pancreatitis in the clinical setting. Is has been largely shown that TNF-α measurements are difficult, because they are substantially hampered by intermittent TNF-α release and a short plasma half-life of less than 20 minutes. Similar observations have been made for IL-1β, which shows an early and transicient increase in most severe cases only [19,50-52]. The soluble TNF receptor complex as well as the IL-1 receptor antagonist (IL-1RA) are more stable than the cytokines itself and thus easier to measure. Although TNF receptors [50,53] and IL-1ra [19,50,51,54] were found to correlate with severe acute pancreatitis and associated organ failure they are no candidate parameters for a meaningful clinical application.

In contrast to the extensive investigations of both cytokines in experimental respect no study has ever been conducted investigating TNF-α or IL-1 antagonism in clinical acute pancreatitis. However, interesting insights can be drawn from a number of sepsis trials. Acute pancreatitis, especially in its severe form, shares striking similarities with sepsis and septic shock. The clinical feature of multi system organ failure and the inflammatory mediator profile are indistinguishable in each of these conditions and suggest a common pathogenic mechanism, albeit as a result of different inflammatory stimuli. Some large randomized multicenter trials on anti TNF-α and IL-1 antagonism in patients with sepsis have demonstrated overall disappointing results. The use of an anti TNF-α antibody in patients with sepsis failed to reduce 28 day mortality in two phase III trials, the North American Sepsis Trial (NORASEPT) and the International Sepsis Trial. Likewise, fusion proteins for p75 TNF-R and p55 TNF-R were ineffective as well [55,56]. A *post hoc* analysis of a controlled trial of human recombinant IL-1 RA in patients with sepsis syndrome revealed an increase in survival time in a subgroup of patients with multi organ failure [57]. However, this observation again could not be confirmed by a subsequent trial [58].

Interleukin-18

Interleukin-18 (IL-18), formerly called interferon-γ-inducing factor, is a novel proinflammatory cytokine playing an important role in the Th-1 response, primarily due to its ability to induce IFN-γ production in T-cells and natural killer cells [59]. IL-18 shares striking similarities with IL-1β concerning structure and function. Both are synthesized as biologically inactive precursors requiring proteolytic cleavage into their mature form by caspases-1/ICE. Moreover, the biological activity of IL-18 is closely related to that of IL-1β: IL-18 induces the gene expression and synthesis of TNF, IL-1, and several chemokines by means of a putative IL-18 receptor complex which is a member of the IL-1R family as well [59]. IL-18 has gained considerable attention since the striking protective effects of caspase-1 inhibition have been reported by a large number of experimental studies in various inflammatory conditions [60] including acute pancreatitis [46-48]. Under therapeutic conditions, caspase-1 antagonism has been more effective in reducing pancreatitis related severity and mortality [48] than has IL-1 antagonism [43,45] in severe experimental models. Therefore, caspase-1 mediated activation of IL-18 may well explain the better results of blocking caspases-1 activity [61]. In fact, by comparing the dynamics of systemic IL-1β and IL-18 concentrations an interesting observation became evident supporting this theory: IL-1β revealed a temporary and moderate increase during the very first days after onset of symptoms in severe disease only [19]. In contrast, IL-18 was released in much higher concentrations with maximum levels during the second week after disease onset in patients with persisting multiorgan system failure [62,63]. The effectiveness of delayed ICE treatment could therefore be a result of inhibited generation of mature IL-18 rather than IL-1β. Interestingly, neutralizing IL-18 activity by monoclonal antibodies has indeed proven to decrease intrapancreatic damage more effectively than neutralizing IL-1β activity in cerulein-induced pancreatitis in mice [64]. Since a therapeutic inhibition of IL-18 has not been investigated in any model of acute pancreatitis so far this interesting cytokine will need further investigation.

Interleukin-6

Interleukin-6 (IL-6) is produced by a wide range of cells including monocytes/macrophages, endothelial cells, and fibroblasts in response to potent proinflammatory stimuli such as endotoxin, IL-1β or TNF-α. IL-6 is the primary inducer of the acute-phase response in various inflammatory conditions [65]. The clinical value of IL-6 for an early and accurate severity stratification of acute pancreatitis has been recognized since the very first reports on cytokine measurements in human acute pancreatitis appeared in the literature [66,67]. Hence, a large number studies have addressed this issue which uniformly confirmed that IL-6 is an earlier marker of severity than the currently established "gold standard" C-reactive protein [19,67]. In terms of predicting pancreatitis associated complications, IL-6 was found to be an excellent predictor of remote organ failure [18,19,53]. In contrast to the exhaustive clinical investigation of IL-6, only few studies have ever addressed the role of this cytokine as potential target for modulating disease severity. From the limited number of experimental studies prophylactic inhibition or genetic deletion of IL-6 had a deleterious [68,69] rather than a protective effect [70] on disease severity and mortality [68-70]. Therefore, the presence of a physiologic IL-6 mediated inflammatory response seems to be necessary for local

and systemic damage control in acute pancreatitis. However, in an overall sense, these observations preclude IL-6 as driving force for the initiation or propagation of organ-specific complications of acute pancreatitis.

Interleukin-10

Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine expressed by almost all cells but primarily released by activated monocytes/macrophages and Th-2 lymphocytes. This cytokine exerts its antiinflammatory properties through inhibition of various proinflammatory cytokines and adhesion molecules on the transcriptional and post-transcriptional level. In addition, IL-10 induces the synthesis of natural cytokine antagonists such as IL-1RA and TNF-α receptors [71]. In human acute pancreatitis, circulating levels of IL-10 were found to correlate with the severity of the disease [19,51] and with organ failure or death in some, but not all of the studies [72,73]. Interesting pathophysiological aspects of this cytokine arose from a number of experimental studies which uniformly demonstrated a protective effect of IL-10 in several models of acute pancreatitis [74-80]. Irrespective whether IL-10 activity was blocked [79], the IL-10 gene was genetically disrupted [78] or the cytokine activity was augmented [74-77,80] organ specific damage in the pancreas, the lung and the liver as well as mortality were significantly reduced. Of specific interest was the observation that the protective effects were still observed when active IL-10 or genetic transfection was started in a therapeutic fashion after acute pancreatitis had been induced [75,76,80].

Convincing experimental evidence of IL-10 modulating approaches has driven the design of clinical trials with the aim to reduce the occurrence of post-ERCP pancreatitis by prophylactic recombinant IL-10 administration. In 2001 Deviere et al. [81] published a single center, double blind controlled study in patients undergoing ERCP, which showed that IL-10 was able to decrease the incidence of post-ERCP pancreatitis independently from other risk factors as well as the length of hospital stay. Inconclusive results came from an US-american trial in which only a trend toward a reduced incidence of post-ERCP pancreatitis and hospital stay was found [82]. A recent meta-analysis including four randomized clinical trials in 294 patients receiving recombinant IL-10 and 259 patients receiving placebo before ERCP could show that IL-10 significantly reduces the risk of post-ERCP pancreatitis [83]. However, the ultimate benefit of IL-10 treatment in preventing post-ERCP pancreatitis is not definitely proven and still needs further evaluation.

Interleukin-2

Increasing clinical evidence suggests that an impaired immune function contributes to the progression of acute pancreatitis. However, cellular immune functions constitute a complex network and seem to have distinct roles in the early toxic and the late septic stages of acute pancreatitis [19,84-86]. Interleukin-2 (IL-2) is a product of activated Th-1 lymphocytes and plays a central role in normal immune function. Clinical and experimental observations have pointed out that the activation of the T-cell system within the inflammatory cascade of acute pancreatitis enhances pancreatic tissue injury [87], the inflammatory response [88-90], and mortality [88]. The release of the soluble IL-2 receptor shows a close correlation with persisting organ complications during the later stages of the disease [19,86] with peak levels predicting a lethal outcome [19]. In diet induced acute pancreatitis in mice a significant reduction of IL-2 production with a consecutively enhanced susceptibility to endotoxin-induced mortality was found during the later stages of the disease which could be reversed by *in vivo* therapy with recombinant IL-2 [90]. These experimental data are well in line with clinical observations and strongly suggest that an impaired immune function increases the risk of subsequent septic complications. Interestingly, the administration on the immunostimulant Levamisole, which is known to potentiate IL-2 production, effectively decreased the incidence of pancreatic infections in a cat model of severe acute pancreatitis [91]. In contrast to the late effects of IL-2 deficiency and immunoparalysis the deleterious consequences of an overt IL-2 mediated T-cell response during the early course of the disease could be emphasized in moderate to severe models of murine acute pancreatitis [87-89]. T and B-cell deficient mice with acute pancreatitis exhibit significantly lower pulmonary damage [88]. The immunosuppressant FK506 which inhibits IL-2 production on the transcriptional level effectively decreased early local and systemic disease severity [89,92], even if given therapeutically after induction of pancreatitis [89]. However, opposite results were shown by another study in which FK506 significantly worsened survival in diet-induced murine pancreatitis [93]. As a result of the obvious controversies even in the experimental setting and the diverse effects of IL-2 during different stages of acute pancreatitis the general concept of immunomodulation as a potential therapeutic target is yet attractive but remains inconclusive and is not ready to be transferred to clinical application.

Platelet-Activating Factor

Platelet-activating factor (PAF) is a lipid that functions as a proinflammatory cytokine since it induces platelet activation and aggregation, neutrophil and monocyte activation, chemotaxis, and vascular effects in terms of vasodilatation and increased vascular permeability [94]. Upon activation leukocytes, platelets, and endothelial cells are major sources of PAF release. PAF synthesis and secretion is closely related to TNF-α and IL-1β in a synergistic manner [94].

A pathophysiological implication of PAF in acute pancreatitis could be first demonstrated by an Italian group in 1989. Administration of PAF into the superior pancreaticoduodenal artery of rabbits induced classical morphologic and biochemical changes of acute pancreatitis in a dose dependent manner within 24-72 hours of injection [95]. These exciting observations have made a number of groups to pursue the role of this novel cytokine in acute pancreatitis [96-103]. Surprisingly, the course of PAF levels has never been investigated in human acute pancreatitis, however, an increase of PAF concentrations in pancreas, lung, ascites, and plasma was found in experimen-

tal models [97,98]. Except one study [103] PAF antagonism has been shown to reduce nearly all pathophysiological changes of acute pancreatitis in established experimental models [99-102]. Besides a significant amelioration of local intrapancreatic damage and microcirculatory derangements a considerable decrease of distant organ involvement and mortality was observed, if PAF antagonists were applied in a therapeutic fashion [99,101,102].

On the basis of the almost uniformly positive experimental results PAF antagonism is one of the few pharmaceutical approaches to acute pancreatitis which have passed the threshold from the experimental setting to clinical application. Lexipafant, one of the most powerful PAF antagonists has been tested in two phase II trials encountering 133 patients with acute pancreatitis [104] or predicted severe acute pancreatitis [105]. In both studies a significant improvement of organ failure or organ failure scores was observed and justified the subsequent initiation of a randomized, double-blind, placebo controlled multicenter trial in 290 patients with predicted severe acute pancreatitis [106]. As with other previous multicenter trials assessing pharmacological agents [13,14] lexipafant did not show any clear benefit in reducing complications, new onset organ failure, or mortality in acute pancreatitis. However, apart from providing interesting insights to the clinical pathophysiology of acute pancreatitis some positive aspects of this approach need to be underscored. Systemic sepsis, development of pseudocysts, and systemic IL-8 and E-selectin levels were significantly lower in the treated than in the non-treated group. A *post hoc* logistic regression analysis showed that initiation of lexipafant treatment within 48 hours of disease onset was related to a lower mortality rate.

Chemokines

Chemokines are a family of small (8-10 kDa), inducible, secreted cytokines with chemotactic and activating effects on different leukocyte subsets thus providing a key stimulus for directing leukocytes to the areas of injury [107]. Over 50 different chemokines and more than 20 receptors with overlapping functions have been characterized. Chemokines can be subdivided on a structural basis into the CXC-subfamily in which the first two of four conserved cysteine residues are separated by another amino acid and the CC-subfamily in which the first two cysteine residues are adjacent. The structural classification of the chemokines also determines their biological activity: while a subgroup of the CXC-chemokines, such as interleukin-8, are potent neutrophil chemoattractants and activators, the CC-chemokines comprising monocyte chemoattractant protein (MCP)-1, -2, -3, macrophage inflammatory protein (MIP)-1α and -1β, regulated on activation, normal T-cell expressed and secreted (RANTES), and eotaxin predominantly affect monocytes [107,108]. Although the importance of chemokines in inflammatory conditions has been well recognized they have only recently become the focus of interest in acute pancreatitis. So far, only a small number of experimental and clinical studies have pointed out that chemokine blockade may be at least as effective as cytokine blockade because of their more proximal position within the inflammatory mediator cascade.

Interleukin-8

Interleukin-8 (IL-8) is the most well known and best characterized member of the chemokine family in acute pancreatitis. IL-8 is synthesized by a large number of different cells such as leukocyte subsets, endothelial and even pancreatic acinar cells [18,23,24]. As a chemokine-specific feature IL-8 is able to stimulate neutrophil chemotaxis and the release of proteolytic enzymes as well as reactive oxygen species thereby enhancing tissue destruction [108]. Along with IL-6, IL-8 has been payed much attention to as early prognostic biochemical variable of disease severity within the first days after onset of symptoms in acute pancreatitis [18,19,109]. An even more interesting aspect of IL-8 was described by our group [110,111]. In patients with necrotizing pancreatitis who developed septic multi organ failure during the later stages of the disease IL-8 has proven as an excellent marker for monitoring this life-threatening complication [111]. Some years later the deleterious role of IL-8 in acute pancreatitis could be nicely demonstrated in the experimental setting [112]. However, only one study has ever investigated the role of anti-IL-8 treatment in this context, yet with interesting results. In a rabbit model of acute pancreatitis Osman et al. [113] could show that prophylactic blockade of IL-8 lead to a significant reduction of systemic severity, lung injury, and mortality, whereas the local intrapancreatic damage remained unchanged. Although it remains yet unproven whether the protective effects are still observed in a therapeutic design, the study strongly supports the role of chemokines in mediating distant organ failure.

Other chemokines

Besides IL-8 other chemokines such as monocyte chemoattractant protein-1 (MCP-1), growth-related oncogene alpha (GRO-α), and epithelial neutrophil-activating protein 78 (ENA78) could be found in high concentrations during the early stages of clinical acute pancreatitis. The quantitative release of these chemokine was more related to the occurrence of systemic than of local complications, thus suggesting a pivotal role in the pathomechanism of distant organ failure [114,115]. In experimental acute pancreatitis chemokines were found to be upregulated as early as 30 minutes after cerulein hyperstimulation [116] and acinar cells were shown to be a major source of chemokine synthesis [23]. In several experimental studies pancreatitis associated pulmonary damage was effectively reduced, if activation of the CXC chemokines CINC or RANTES *via* specific antibodies or synthetic inhibitors was blocked [117,118]. Targeted disruption of the MIP-1α/RANTES receptor CCR-1 had a similar effect in the cerulein model in mice [119]. An interesting common observation of these studies was the fact that despite a significant reduction of overall severity and pulmonary damage no effects on local intrapancreatic damage were observed. So far, MCP-1 seems to be only chemokine which exerts a detrimental role on the degree of local intrapancreatic damage [120]. Unfortunately, although the protective effects were still observed after therapeutic inhibition in most studies [117,118,120], the role of chemokine antagonism on mortality has never been investigated so far.

Anti cytokine strategies in the clinical setting

A large body of experimental evidence suggests that modulation of pro- or antiinflammatory cytokine activation has favourable effects on local and systemic disease severity in acute pancreatitis. Different approaches to inhibit cytokine activation have been used in various experimental models, many of them have even proven effective, if applied in a therapeutic fashion after induction of acute pancreatitis. Despite the number of favourable experimental studies, only few clinical trials on anti-cytokine strategies have been performed. Hence, only PAF antagonism and IL-10 treatment have been investigated in acute pancreatitis by controlled studies with largely disappointing results.

Yet, cytokines are still an exciting and challenging target for potential new approaches to the treatment of acute pancreatitis. The failure of the few representative clinical studies on anti--cytokine strategies does not necessarily mean that this approach is generally ineffective since we know that those cytokine identified so far most likely represent the "tip of the iceberg" only [121]. However, before new clinical trials are started, there must be careful consideration of why previous interventions were not effective.

In fact, every "first order" cytokine known so far is a strong promoter for the progression of acute pancreatitis on its own. However, a magnitude of other potent inflammatory mediators is known to interact and control the cytokine release and vice versa. It therefore should be kept in mind that the concept of blocking a single elevated cytokine may be too simple to deal with the complex problem of acute pancreatitis. It remains largely questionable, if there is any "ultimate" target at all, and if so it still needs to be defined.

Secondly, as patients with acute pancreatitis move through different phases from sterile inflammatory response to septic organ failure, there may be intervals when it is appropriate to inhibit multiple cytokines while at other times it may be appropriate to augment them.

A third point involves the optimum timing to start cytokine antagonism. Norman et al. [16] has well described that the therapeutic window in acute pancreatitis is restricted to about 48-72 hours following the onset of symptoms until complications such as necrosis or organ failure develop. This concept is supported by at least two controlled trials using different pharmaceutical approaches: the European PAF-antagonist phase III trial [106] and the German Octreotide trial [122] in which a beneficial effect was achieved when treatment was started within 48 hours after onset of symptoms. However, clinical experience has shown that many patients with severe disease usually present to specialized centers capable to provide this kind of specific and expensive treatment beyond the 48 hour time interval after onset of symptoms.

At present, a truly optimistic view on anti-cytokine strategies is not supported by any of the large representative clinical studies. The main limitations of anti-cytokine-treatment strategies relate to the difficulties in translating successful experimental observations into reproducible clinical associations due to the complexity and individuality of the human nature [123]. Moreover, deficiencies in study design with insufficient sample sizes, inconsistent definitions and tools to stratify disease severity as well as non-comparable study endpoints have further contributed to the failure of nearly all clinical studies on new pharmacological approaches to acute pancreatitis. Despite all limitations, treatment of acute pancreatitis by cytokine modulation still remains an attractive concept. However, further work will be needed to overcome the fundamental conceptional problems as well as to accomplish our still incomplete understanding of the complex pathophysiology of this challenging disease.

References

1. Bradley EL 3rd. A clinically based classification system for acute pancreatitis. Arch Surg, 1993; 128: 586-90.
2. Beger HG, Bittner R, Block S, Büchler M. Bacterial contamination of pancreatic necrosis. A prospective clinical study. Gastroenterol, 1986, 49: 433-8.
3. 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: 1020-4.
4. Isenmann R, Rau B, Beger HG. Early severe acute pancreatitis: characteristics of a new subgroup. Pancreas, 2001; 22: 274-8.
5. Buter A, Imrie CW, Carter CR, Evans S, McKay CJ. Dynamic nature of early organ dysfunction determines outcome in acute pancreatitis. Br J Surg, 2002; 89: 298-302.
6. Johnson CD, Abu-Hilal M. Persistent organ failure during the first week as a marker of fatal outcome in acute pancreatitis. Gut, 2004; 53: 1340-4.
7. Working Party of the British Society of Gastroenterology; Association of Surgeons of Great Britain and Ireland; Pancreatic Society of Great Britain and Ireland; Association of Upper GI Surgeons of Great Britain and Ireland: UK guidelines for the management of acute pancreatitis. Gut, 2005; 54 (Suppl. 3): 1-9.
8. 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, Buchler MW. IAP guidelines for the surgical management of acute pancreatitis. Pancreatology, 2002; 2: 565-73.
9. Chiari H. Über die Selbstverdaung des menschlichen Pankreas. Zeitschrift für Heilkunde, 1896; 17: 69-96.
10. Gorelick FS, Otani T. Mechanisms of intracellular zymogen activation. Baillieres Best Pract Res Clin Gastroenterology, 1999; 13: 227-40.
11. Frossard JL. Trypsin activation peptide (TAP) in acute pancreatitis: from pathophysiology to clinical usefulness. JOP, 2001; 2: 69-77.
12. Halangk W, Krüger B, Ruthenbürger M, Stürzebecher J, Albrecht E, Lippert H, Lerch MM. Trypsin activity is not involved in premature, intrapancreatic trypsinogen activation. Am J Physiol Gastrointest Liver Physiol, 2002; 282: G367-74.
13. Seta T, Noguchi Y, Shimada T, Shikata S, Fukui T. Treatment of acute pancreatitis with protease inhibitors: A meta-analysis. Eur J Gastroenterol Hepatol, 2004; 16: 1287-93.
14. Cavallini G, Frulloni L. Somatostatin and octreotide in acute pancreatitis: the never-ending story. Dig Liver Dis, 2001; 33: 192-201.
15. Rinderknecht H. Fatal pancreatitis, a consequence of excessive leukocyte stimulation? Int J Pancreatol, 1988; 3: 105-12.
16. Norman J. The role of cytokines in the pathogenesis of acute pancreatitis. Am J Surg, 1998; 175: 76-83.
17. Makhija R, Kingsnorth AN. Cytokine storm in acute pancreatitis. J Hepatobiliary Pancreat Surg, 2002; 9: 401-10.
18. McKay CJ, Gallagher G, Brooks B, Imrie CW, Baxter JN. Increased monocyte cytokine production in association with systemic complications in acute pancreatitis. Br J Surg, 1996; 83: 919-23.
19. Mayer J, Rau B, Gansauge F, Beger HG. Inflammatory mediators in human acute pancreatitis: clinical and pathophysiological implications. Gut, 2000; 47: 546-52.
20. Rau B, Schilling MK, Beger HG. Laboratory markers of severe acute pancreatitis. Dig Dis, 2004; 22: 247-57.
21. Fink GW, Norman JG. Intrapancreatic interleukin-1 beta gene expression by specific leukocyte populations during acute pancreatitis. J Surg Res, 1996; 63: 369-73.
22. 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: 1853-62.

23. Blinman TA, Gukovsky I, Mouria M, Zaninovic V, Livingston E, Pandol SJ, Gukovskaya AS. Activation of pancreatic acinar cells on isolation from tissue: cytokine upregulation via p38 MAP kinase. Am J Physiol Cell Physiol, 2000; 279: C1993-2003.

24. Blanchard JA 2nd, Barve S, Joshi-Barve S, Talwalker R, Gates LK Jr. Cytokine production by CAPAN-1 and CAPAN-2 cell lines. Dig Dis Sci, 2000; 45: 927-32.

25. Ramudo L, Manso MA, Sevillano S, de Dios I. Kinetic study of TNF-alpha production and its regulatory mechanisms in acinar cells during acute pancreatitis induced by bile-pancreatic duct obstruction. J Pathol, 2005; 206: 9-16.

26. Exley AR, Leese T, Holliday MP, Swann RA, Cohen J. Endotoxaemia and serum tumour necrosis factor as prognostic markers in severe acute pancreatitis. Gut, 1992; 33: 1126-8.

27. Grewal HP, Kotb M, el Din AM, Ohman M, Salem A, Gaber L, Gaber AO. Induction of tumor necrosis factor in severe acute pancreatitis and its subsequent reduction after hepatic passage. Surgery, 1994; 115: 213-21.

28. Hughes CB, Gaber LW, Kotb M, Mohey el-Din AB, Pabst M, Gaber AO. Induction of acute pancreatitis in germ-free rats: evidence of a primary role for tumor necrosis factor-alpha. Surgery, 1995; 117: 201-5.

29. Norman JG, Fink GW, Franz MG. Acute pancreatitis induces intrapancreatic tumor necrosis factor gene expression. Arch Surg, 1995; 130: 966-70.

30. Hughes CB, Henry J, Kotb M, Lobaschevsky A, Sabek O, Gaber AO. Up-regulation of TNF alpha mRNA in the rat spleen following induction of acute pancreatitis. J Surg Res, 1995; 59: 687-93.

31. Norman JG, Fink GW, Denham W, Yang J, Carter G, Sexton C, Falkner J, Gower WR, Franz MG. Tissue-specific cytokine production during experimental acute pancreatitis. A probable mechanism for distant organ dysfunction. Dig Dis Sci, 1997; 42: 1783-8.

32. Grewal HP, Mohey el Din A, Gaber L, Kotb M, Gaber AO. Amelioration of the physiologic and biochemical changes of acute pancreatitis using an anti-TNF-alpha polyclonal antibody. Am J Surg, 1994; 167: 214-8.

33. Hughes CB, Gaber LW, Mohey el-Din AB, Grewal HP, Kotb M, Mann L, Gaber AO. Inhibition of TNF alpha improves survival in an experimental model of acute pancreatitis. Am J Surg, 1996; 62: 8-13.

34. Hughes CB, Grewal HP, Gaber LW, Kotb M, el-Din AB, Mann L, Gaber AO. Anti-TNF alpha therapy improves survival and ameliorates the pathophysiologic sequelae in acute pancreatitis in the rat. Am J Surg, 1996; 171: 274-80.

35. Norman JG, Fink GW, Messina J, Carter G, Franz MG. Timing of tumor necrosis factor antagonism is critical in determining outcome in murine lethal acute pancreatitis. Surgery, 1996; 120: 515-21.

36. Denham W, Yang J, Fink G, Denham D, Carter G, Ward K, Norman J. Gene targeting demonstrates additive detrimental effects of interleukin-1 and tumor necrosis factor during pancreatitis. Gastroenterol, 1997; 113: 1741-6.

37. Yang J, Murphy C, Denham W, Botchkina G, Tracey KJ, Norman J. Evidence of a central role for p38 map kinase induction of tumor necrosis factor alpha in pancreatitis-associated pulmonary injury. Surgery, 1999; 126: 216-22.

38. Jaffray C, Yang J, Carter G, Mendez C, Norman J. Pancreatic elastase activates pulmonary nuclear factor kappa B and inhibitory kappa B, mimicking pancreatitis-associated adult respiratory distress syndrome. Surgery, 2000; 128: 225-31.

39. Fink GW, Norman JG. Specific changes in the pancreatic expression of the interleukin 1 family of genes during experimental acute pancreatitis. Cytokine, 1997; 9: 1023-7.

40. Fink G, Yang J, Carter G, Norman J. Acute pancreatitis-induced enzyme release and necrosis are attenuated by IL-1 antagonism through an indirect mechanism. J Surg Res, 1997; 67: 94-7.

41. Denham W, Yang J, Fink G, Denham D, Carter G, Bowers V, Norman J. TNF but not IL-1 decreases pancreatic acinar cell survival without affecting exocrine function: a study in the perfused human pancreas. J Surg Res, 1998; 74: 3-7.

42. Tanaka N, Murata A, Uda K, Toda H, Kato T, Hayashida H, Matsuura N, Mori T. Interleukin-1 receptor antagonist modifies the changes in vital organs by acute necrotizing pancreatitis in a rat experimental model. Crit Care Med, 1995; 23: 901-8.

43. Norman J, Franz M, Messina J, Riker A, Fabri PJ, Rosemurgy AS, Gower WR Jr. Interleukin-1 receptor antagonist decreases severity of experimental acute pancreatitis. Surgery, 1995; 117: 648-55.

44. Norman JG, Fink G, Franz M, Guffey J, Carter G, Davison B, Sexton C, Glaccum M. Active interleukin 1 receptor required for maximal progression of acute pancreatitis. Ann Surg, 1996; 223: 163-9.

45. Norman JG, Franz MG, Fink GS, Messina J, Fabri PJ, Gower WR, Carey LC. Decreased mortality of severe acute pancreatitis after proximal cytokine blockade. Ann Surg, 1995; 221: 625-34.

46. Norman J, Yang J, Fink G, Carter G, Ku G, Denham W, Livingston D. Severity and mortality of experimental pancreatitis are dependent on interleukin-1 converting enzyme. J Interferon Cytokine Res, 1997; 17: 113-8.

47. Rau B, Paszkowski AS, Lillich S, Baumgart K, Möller P, Beger HG. Differential effects of caspase-1/interleukin-1beta-converting enzyme on acinar cell necrosis and apoptosis in severe acute experimental pancreatitis. Lab Invest, 2001; 81: 1001-13.

48. Paszkowski AS, Rau B, Mayer JM, Möller P, Beger HG. Therapeutic application of caspase-1/interleukin-1β-converting enzyme inhibitor decreases the death rate of severe acute experimental pancreatitis. Ann Surg, 2002; 235: 68-76.

49. Denham W, Fink G, Yang J, Ulrich P, Tracey K, Norman J. Small molecule inhibition of tumor necrosis factor gene processing during acute pancreatitis prevents cytokine cascade progression and attenuates pancreatitis severity. Am Surg, 1997; 63: 1045-9.

50. Dugernier TL, Laterre PF, Wittebole X, Roeseler J, Latinne D, Reynaert MS, Pugin J. Compartmentalization of the inflammatory response during acute pancreatitis. Correlation with local and systemic complications. Am J Resp Crit Care Med, 2003; 168: 148-57.

51. Brivet FG, Emilie D, Galanaud P. Pro- and anti-inflammatory cytokines during acute severe pancreatitis: an early and sustained response, although unpredictable of death. Crit Care Med, 1999; 27: 749-55.

52. Chen CC, Wang SS, Lee FY, Chang FY, Lee SD. Proinflammatory cytokines in early assessment of the prognosis of acute pancreatitis. Am J Gastroenterol, 1999; 94: 213-8.

53. de Beaux AC, Goldie AS, Ross JA, Carter DC, Fearon KC. Serum concentrations of inflammatory mediators related to organ failure in patients with acute pancreatitis. Br J Surg, 1996; 83: 349-53.

54. Mentula P, Kylanpaa ML, Kemppainen E, Jansson SE, Sarna S, Puolakkainen P, Haapiainen R, Repo H. Plasma anti-inflammatory cytokines and monocyte human leucocyte antigen-DR expression in patients with acute pancreatitis. Scand J Gastroenterol, 2004; 39: 178-87.

55. Cohen J, Carlet J. INTERSEPT: an international, multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor-alpha in patients with sepsis. International Sepsis Trial Study Group. Crit Care Med, 1996; 24: 1431-40.

56. Abraham E, Anzueto A, Gutierrez G, Tessler S, San Pedro G, Wunderink R, Dal Nogare A, Nasraway S, Berman S, Cooney R, Levy H, Baughman R, Rumbak M, Light RB, Poole L, Allred R, Constant J, Pennington J, Porter S. Double-blind randomised controlled trial of monoclonal antibody to human tumor necrosis factor in treatment of septic shock. NORASEPT II Study Group. Lancet, 1998; 351: 929-33.

57. Fisher CJ Jr, Dhainaut JF, Opal SM, Pribble JP, Balk RA, Slotman GJ, Iberti TJ, Rackow EC, Shapiro MJ, Greenman RL. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. JAMA, 1994; 271: 1836-43.

58. Opal SM, Fisher CJ Jr, Dhainaut JF, Vincent JL, Brase R, Lowry SF, Sadoff JC, Slotman GJ, Levy H, Balk RA, Shelly MP, Pribble JP, LaBrecque JF, Lookabaugh J, Donovan H, Dubin H, Baughman R, Norman J, DeMaria E, Matzel K, Abraham E, Seneff M. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. Crit Care Med, 1997; 25: 1115-24.

59. Dinarello CA. IL-18: a Th1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol, 1999; 103: 11-24.

60. Siegmund B. Interleukin-1beta converting enzyme (caspase-1) in intestinal inflammation. Biochem Pharmacol, 2002; 64: 1-8.

61. Dinarello CA, Fantuzzi G. Interleukin-18 and host defense against infection. J Infect Dis, 2003; 187: S370-84.

62. Rau B, Baumgart K, Paszkowski AS, Mayer JM, Beger HG. Clinical relevance of caspase-1 activated cytokines in acute pancreatitis: High correlation of serum interleukin-18 with pancreatic necrosis and systemic complications. Crit Care Med, 2001; 29: 1556-62.

63. Wereszczynska-Siemiatkowska U, Mroczko B, Siemiatkowski A. Serum profiles of interleukin-18 in different severity forms of human acute pancreatitis. Scand J Gastroenterol, 2002; 37: 1097-102.

64. Rau B, Zhuang Y, Schöler C, Hans J, Tilton B, Schilling M. Caspase-1 activated cytokines in experimental acute pancreatitis: synergistic effects of IL-1ß and IL-18 on the development of disease severity. Pancreas, 2003; 27: 403.

65. Gadient RA, Patterson PH. Leukemia inhibitory factor, Interleukin 6, and other cytokines using the GP130 transducing receptor: roles in inflammation and injury. Stem Cells, 1999; 17: 127-37.

66. Leser HG, Gross V, Scheibenbogen C, Heinisch A, Salm R, Lausen M, Ruckauer K, Andreesen R, Farthmann EH, Scholmerich J. Elevation of serum interleukin-6 concentration precedes acute-phase response and reflects severity in acute pancreatitis. Gastroenterol, 1991; 101: 782-5.

67. Heath DI, Cruickshank A, Gudgeon M, Jehanli A, Shenkin A, Imrie CW. Role of interleukin-6 in mediating the acute phase protein response and potential as an early means of severity assessment in acute pancreatitis. Gut, 1993; 34: 41-5.

68. Liu Q, Djuricin G, Nathan C, Gattuso P, Weinstein RA, Prinz RA. The effect of interleukin-6 on bacterial translocation in acute canine pancreatitis. Int J Pancreatol, 2000; 27: 157-65.

69. Cuzzocrea S, Mazzon E, Dugo L, Centorrino T, Ciccolo A, McDonald MC, de Sarro A, Caputi AP, Thiemermann C. Absence of endogenous interleukin-6 enhances the inflammatory response during acute pancreatitis induced by cerulein in mice. Cytokine, 2002; 18: 274-85.

70. Suzuki S, Miyasaka K, Jimi A, Funakoshi A. Induction of acute pancreatitis by cerulein in human IL-6 gene transgenic mice. Pancreas, 2000; 21: 86-92.

71. Morre KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol, 2001; 19: 683-765.

72. Simovic MO, Bonham MJ, Abu-Zidan FM, Windsor JA. Anti-inflammatory cytokine response and clinical outcome in acute pancreatitis. Crit Care Med, 1999; 27: 2662-5.

73. Mentula P, Kylanpaa ML, Kemppainen E, Jansson SE, Sarna S, Puolakkainen P, Haapiainen R, Repo H. Early prediction of organ failure by combined markers in patients with acute pancreatitis. Br J Surg, 2005; 92: 68-75.

74. Van Laethem JL, Marchant A, Delvaux A, Goldman M, Robberecht P, Velu T, Deviere J. Interleukin 10 prevents necrosis in murine experimental acute pancreatitis. Gastroenterology, 1995; 108: 1917-22.

75. Kusske AM, Rongione AJ, Ashley SW, McFadden DW, Reber HA. Interleukin-10 prevents death in lethal necrotizing pancreatitis in mice. Surgery, 1996; 120: 284-8.

76. Rongione AJ, Kusske AM, Kwan K, Ashley SW, Reber HA, McFadden DW. Interleukin 10 reduces the severity of acute pancreatitis in rats. Gastroenterology, 1997; 112: 960-7.

77. Osman MO, Jacobsen NO, Kristensen JU, Deleuran B, Gesser B, Larsen CG, Jensen SL. IT 9302, a synthetic interleukin-10 agonist, diminishes acute lung injury in rabbits with acute necrotizing pancreatitis. Surgery, 1998; 124: 584-92.

78. Gloor B, Todd KE, Lane JS, Rigberg DA, Reber HA Mechanism of increased lung injury after acute pancreatitis in IL-10 knockout mice. J Surg Res, 1998; 80: 110-4.

79. Van Laethem JL, Eskinazi R, Louis H, Rickaert F, Robberecht P, Deviere J. Multisystemic production of interleukin 10 limits the severity of acute pancreatitis in mice. Gut, 1998; 43: 408-13.

80. Zou WG, Wang DS, Lang MF, Jin DY, Xu DH, Zheng ZC, Wu ZH, Liu XY. Human interleukin 10 gene therapy decreases the severity and mortality of lethal pancreatitis in rats. J Surg Res, 2002; 103: 121-6.

81. Deviere J, Le Moine O, Van Laethem JL, Eisendrath P, Ghilain A, Severs N, Cohard M. Interleukin 10 reduces the incidence of pancreatitis after therapeutic endoscopic retrograde cholangiopancreatography. Gastroenterology, 2001; 120: 498-505.

82. Dumot JA, Conwell DL, Zuccaro G Jr, Vargo JJ, Shay SS, Easley KA, Ponsky JL. A randomized, double blind study of interleukin 10 for the prevention of ERCP-induced pancreatitis. Am J Gastroenterol 2001; 96: 2098-102.

83. Singh et al. DDW 2002, abstract T 1726.

84. Curley PJ. Endotoxin, cellular immune dysfunction and acute pancreatitis. Ann R Coll Surg Engl, 1996; 78: 531-5.

85. Kylanpaa-Back ML, Takala A, Kemppainen E, Puolakkainen P, Kautiainen H, Jansson SE, Haapiainen R, Repo H. Cellular markers of systemic inflammation and immune suppression in patients with organ failure due to severe acute pancreatitis. Scand J Gastroenterol, 2001; 36: 1100-7.

86. Kylanpaa-Back ML, Takala A, Kemppainen EA, Puolakkainen PA, Leppaniemi AK, Karonen SL, Orpana A, Haapiainen RK, Repo H. Procalcitonin, soluble interleukin-2 receptor, and soluble E-selectin in predicting the severity of acute pancreatitis. Crit Care Med, 2001; 29: 63-9.

87. Demols A, Le Moine O, Desalle F, Quertinmont E, Van Laethem JL, Deviere J. DC4(+) T cells play an important role in acute experimental pancreatitis in mice. Gastroenterology, 2000; 118: 582-90.

88. Mayer J, Laine VJO, Rau B, Hotz HG, Foitzik T, Nevalainen TJ, Beger HG. Systemic lymphocyte activation modulates the severity of diet-induced acute pancreatitis in mice. Pancreas, 1999; 19: 62-8.

89. Mayer JM, Laine VJ, Gezgin A, Kolodziej S, Nevalainen TJ, Storck M, Beger HG. Single doses of FK506 and OKT3 reduce severity in early experimental acute pancreatitis. Eur J Surg, 2000; 166: 734-41.

90. Curley P, Nestor M, Collins K, Saporoschetz I, Mendez M, Mannick JA, Rodrick ML. Decreased interleukin-2 production in murine acute pancreatitis: potential for immunomodulation. Gastroenterology, 1996; 110: 583-8.

91. Widdison AL, Karanjia ND, Alvarez C, Reber HA. Influence of levamisole on pancreatic infection in acute pancreatitis. Am J Surg, 1992; 163: 100-3.

92. Rau B, Paszkowski AS, Lillich S, Mayer JM, Möller P, Beger HG. Effects of FK506 on pancreatic acinar cell damage and mortality in severe acute experimental pancreatitis. Pancreas, 2001; 23: 457.

93. Echigo Y, Inoue K, Kogire M, Doi R, Higashide S, Sumi S, Kaji H, Imamura M. Effects of Cyclosporine and Tacrolimus (FK 506) on acute pancreatitis in mice. Arch Surg, 1995; 130: 64-8.

94. Johnson CD. Platelet-activating factor and platelet-activating factor antagonists in acute pancreatitis. Dig Surg, 1999; 16: 93-101.

95. Emanuelli G, Montrucchio G, Gaia E, Dughera L, Corvetti G, Gubetta L. Experimental acute pancreatitis induced by platelet activating factor in rabbits. Am J Pathol, 1989; 134: 315-26.

96. Dabrowski A, Gabryelewicz A, Chyczewski L. The effect of platelet activating factor antagonist (BN 52021) on cerulein-induced acute pancreatitis with reference to oxygen radicals. Int J Pancreatol, 1991; 8: 1-11.

97. Zhou W, McCollum MO, Levine BA, Olson MS. Role of platelet-activating factor in pancreatitis-associated acute lung injury in the rat. Am J Pathol, 1992; 140: 971-9.

98. Kald B, Kald A, Ihse I, Tagesson C. Release of platelet-activating factor in acute experimental pancreatitis. Pancreas, 1993; 8: 440-2.

99. Formela LJ, Wood LM, Whittaker M, Kingsnorth AN. Amelioration of experimental acute pancreatitis with a potent platelet-activating factor antagonist. Br J Surg, 1994; 81: 1783-5.

100. Wang X, Sun Z, Borjesson A, Haraldsen P, Aldman M, Deng X, Leveau P, Andersson R. Treatment with lexipafant ameliorates the severity of pancreatic microvascular endothelial barrier dysfunction in rats with acute hemorrhagic pancreatitis. Int J Pancreatol, 1999; 25: 45-52.

101. Hofbauer B, Saluja AK, Bhatia M, Frossard JL, Lee HS, Bhagat L, Steer ML. Effect of recombinant platelet-activating factor acetylhydrolase on two models of experimental acute pancreatitis. Gastroenterology, 1998; 115: 1238-47.

102. Foitzik T, Hotz HG, Eibl G, Hotz B, Kirchengast M, Buhr HJ. Therapy for microcirculatory disorders in severe acute pancreatitis: effectiveness of platelet-activating factor receptor blockade vs. endothelin receptor blockade. J Gastrointest Surg, 1999; 3: 244-51.

103. Rivera JA, Werner J, Warshaw AL, Lewandrowski KB, Rattner DW, Fernandez del Castillo C. Lexipafant fails to improve survival in severe necrotizing pancreatitis in rats. Int J Pancreatol, 1998; 23: 101-6.

104. Kingsnorth AN, Galloway SW, Formela LJ. Randomized, double-blind phase II trial of Lexipafant, a platelet-activating factor antagonist, in human acute pancreatitis. Br J Surg, 1995; 82: 1414-20.

105. McKay CJ, Curran F, Sharples C, Baxter JN, Imrie CW. Prospective placebo-controlled randomized trial of lexipafant in predicted severe acute pancreatitis Br J Surg, 1997; 84: 1239-43.

106. Johnson CD, Kingsnorth AN, Imrie CW, McMahon MJ, Neoptolemos JP, McKay C, Toh SK, Skaife P, Leeder PC, Wilson P, Larvin M, Curtis LD. Double blind, randomised, placebo controlled study of a platelet activating factor antagonist, lexipafant, in the treatment and prevention of organ failure in predicted severe acute pancreatitis. Gut, 2001; 48: 62-9.

107. Rollins BJ. Chemokines. Blood, 1997; 90, 909-28.

108. Baggiolini M, Loetscher P, Moser B. Interleukin-8 and the chemokine family. Int J Immunopharmacol, 1995; 17: 103-8.

109. Gross V, Andreesen R, Leser H-G, Ceska M, Liehl E, Lausen M, Farthmann EH, Schölmerich J. Interleukin-8 and neutrophil activation in acute pancreatitis. Eur J Clin Invest, 1992; 22: 200-3.

110. Rau B, Steinbach G, Gansauge F, Mayer JM, Grünert A, Beger HG. The potential role of procalcitonin and interleukin-8 in the

prediction of infected necrosis in acute pancreatitis. Gut, 1997; 41: 832-40.

111. Rau B, Steinbach G, Gansauge F, Mayer JM, Grünert A, Beger HG. The role of Interleukin-8 in the severity assessment of septic complications in necrotizing pancreatitis. Digestion, 1997; 58: 11.

112. Osman MO, Lausten SB, Jakobsen NO, Kristensen JU, Deleuran B, Larsen CG, Jensen SL. Graded experimental acute pancreatitis: monitoring of a renewed rabbit model focusing on the production of interleukin-8 (IL-8) and CD11b/CD18. Eur J Gastroenterol Hepatol, 1999; 11: 137-49.

113. Osman MO, Kristensen JU, Jacobsen NO, Lausten SB, Deleuran B, Deleuran M, Gesser B, Matsushima K, Larsen CG, Jensen SL. A monoclonal anti-interleukin 8 antibody (WS-4) inhibits cytokine response and acute lung injury in experimental severe acute necrotising pancreatitis in rabbits. Gut, 1998; 43: 232-9.

114. Shokuhi S, Bhatia M, Christmas S, Sutton R, Neoptolemos JP, Slavin J. Levels of the chemokines growth-related oncogene alpha and epithelial neutrophil-activating protein 78 are raised in patients with severe acute pancreatitis. Br J Surg, 2002; 89: 566-72.

115. Rau B, Baumgart K, Kruger CM, Schilling M, Beger HG. CC-chemokine activation in acute pancreatitis: enhanced release of monocyte chemoattractant protein-1 in patients with local and systemic complications. Intensive Care Med, 2003; 29: 622-9.

116. Grady T, Liang P, Ernst SA, Logsdon CD. Chemokine gene expression in rat pancreatic acinar cells is an early event associated with acute pancreatitis. Gastroenterol, 1997; 113, 1966-75.

117. Bhatia M, Brady M, Zagorski J, Christmas SE, Campbell F, Neoptolemos JP, Slavin J. Treatment with neutralizing antibody against cytokine-induced neutrophil chemoattractant (CINC) protects rats against acute pancreatitis associated lung injury. Gut, 2000; 47: 838-44.

118. Bhatia M, Proudfoot AE, Wells TN, Christmas S, Neoptolemos JP, Slavin J. Treatment with Met-RANTES reduces lung injury in caerulein-induced pancreatitis. Br J Surg, 2003; 90: 698-704.

119. Gerard C, Frossard JL, Bhatia M, Saluja A, Gerard NP, Lu B, Steer ML. Trageted disruption of the β-chemokine receptor CCR-1 protects against pancreatitis associated lung injury. J Clin Invest, 1997; 100: 2022-7.

120. Bhatia M, Ramnath RD, Chevali L, Guglielmotti A. Treatment with bindarit, a blocker of MCP-1 synthesis, protects mice against acute pancreatitis. Am J Physiol Gastrointest Liver Physiol, 2005; 288: G1259-65.

121. Matuschak GM. Circulating cytokine concentrations and outcome prediction in intensive care unit patients: still the tip of the iceberg? Crit Care Med, 1996; 24: 1769-71.

122. Uhl W, Büchler MW, Malfertheiner P, Beger HG, Adler G, Gaus W. A randomised, double blind, multicentre trial of octreotide in moderate to severe acute pancreatitis. Gut, 1999; 45: 97-104.

123. Imahara SD, O'Keefe GE. Genetic determinants of the inflammatory response. Curr Opin Crit Care, 2004; 10: 318-24.