Cytokines in Chronic Pancreatitis and Pancreatic Cancer

M. Shamsutdinova1, R. Timme2, Sh. Zakirkhodjaev2, G. Sadriddinova1.

1 Clinic of Internal Medicine II, University Hospital, Freiburg, Germany.
2 Department of Internal Medicine, Haematology, Tashkent Medical Academy, Republic of Uzbekistan

INTRODUCTION

In recent years, the interest of clinicians in chronic pancreatitis (CP) has increased due to the better understanding of the pathogenesis of CP and pancreatic cancer (PC). The role of cytokines as an important link in the regulation of inflammation and pancreas regeneration in patients with CP remains unclear. All pro-inflammatory cytokines examined in this study (IL-2R, IL-6, TNF-α) have been shown to be produced within the pancreas during the early stages of pancreatitis and show elevated levels in both blood and pancreatic tissue. Serum albumin levels were reduced in 21 (51%) patients. The tumor markers CA 19-9 was elevated in 15 (38%) patients with a tumor in the head of the pancreas that was confirmed by computer tomography.

CONCLUSION. The results indicate that cytokines are activated in patients with CP and PC respectively. This activation is accompanied by increased levels of pro-inflammatory cytokines IL-2R, IL-6, TNF-α, and INF-γ, which closely correlate with the major pathological clinical and laboratory parameters in these patients.

Keywords: Chronic pancreatitis, Cytokines, Pancreas Carcinoma, Pathogenesis.
TNF-α was found to influence each of the three parameters of PSCs activation examined in this study, namely α-SMA expression, cell proliferation, and collagen synthesis. The effect of TNF-α on α-SMA expression concurs with the findings reported with hepatic stellate cells (HSCs). The observed mitogenic effect of TNF-α on PSCs in vitro represents an important finding. Recent in vivo studies using animal models of pancreatic necrosis inflammation and fibrosis have reported an increase in the number of PSCs in fibrotic areas of the pancreas. Given the results of this study, it is possible that TNF-α, either alone or together with other known stellate cell mitogens such as pancreatic ductal growth factor, mediates stellate cell proliferation under conditions of pancreatic injury. [1]

In addition to its pro-inflammatory effects, IL-6 is known to have many anti-inflammatory effects (including down regulation of TNF-α and IL-2R, and induction of adrenocorticotrophic hormone and glucocorticoid synthesis). In vivo, IL-6 is produced in response to increased TNF-α and IL-2R secretion in many cell types. Indeed, PSCs have been shown to synthesize IL-6 when activated by TNF-α, IL-2R, or bacterial endotoxins. The possibility of IL-6 production by PSCs has not yet been examined. Nevertheless, the findings of this study suggest that IL-6, whatever its source, has the potential to act as a regulatory factor for the activation of HSCs during pancreatic injury in vivo. Recent studies demonstrated a prognostic link between the level of IL-2R, IL-6 and TNF-α the severity of acute pancreatitis, suggesting a potential link between these inflammatory cytokines and the tumor risk.[1,2] However, the molecular targeted therapy of pancreas cancer is still at a conceptual level. The selective inhibition of IL-6 secretion by targeting specific integrin has been shown to significantly reduce metastasis but did not result in complete arrest of tumor growth.

Similar studies suggested that the therapeutic targeting of cytokines, which are directly involved in establishing the tumor microenvironment, serves as an essential convergence point for multiple cytokine pathways. The complex interaction of cytokines with pancreatic cancer cell-signalling pathways and the multitude of chemical cross talks between microenvironment and tumor make it unlikely that a single molecular target will be effective. Rather, the most effective treatment will be one that combines multiple molecular therapeutic targets as well as the based on chemokine tumor markers. In this context, previous studies have shown a direct correlation between IL-6 levels, tumor growth, and antigenic potential. An increased level of IL-6 is in part directly responsible for the oncogenic activity and aggressive metastasis seen in pancreatic tumors. These data indicate that IL-2R and IL-6 play a critical role in tumor pathogenesis and may be useful for the early diagnosis of pancreas cancer, using for example IL-6 as a marker. Cytokines are very complex interacting molecular signals, which are involved in the regulation of other. The aim of the study was the determination of the levels of standard laboratory parameters and of serum cytokines, respectively in patients with CP and PC.

**MATERIALS AND METHODS**

40 patients with CP (12 men, 28 women) aged between 29 and 65 years (average 45±18 years) were examined in our clinic. The clinical duration of CP was 13±6 years. 15 patients with PC (8 men, 7 women) aged between and years (average 58±15 years) were included in the study. The control group consisted of 25 healthy volunteers (8 men, 17 women) with an average age of 38±15 years. The causes of CP were viral infections, alcohol, metabolic, or autoimmune diseases and others. In all patients the standard laboratory parameters amylase, lipase, AST, ALT, LDH, CRP and cancer-specific markers CA 19-9 were determined. In addition, the serum levels of TNF-α, IL-2R, IL-6, and INF-γ were determined enzyme immune assays.

**RESULTS**

30 (75%) patients with CP were overweight (BMI = 37.3 ± 4 kg/m²) and 10 (25%) patients were asthenic. Non-insulin dependent diabetes mellitus was diagnosed in 14 (35%) patients, gastric dyspepsia in 6 (15%) patients.

Clinical and laboratory analyses revealed an enlarged pancreas in 32 (80%), and an enlarged spleen in 3 (7%) patients, respectively. Bilirubin was elevated in 17 (41%) patients. Serum lipase, γ-glutamyl trans peptidase (γ-GT) and alkaline phosphatase (AP) activities were significantly elevated in 15 (62%) patients. ALT and AST activities were significantly increased in 12 (50%), moderately elevated (3-5 x ULN) in 6 (15%) and slightly elevated (1.5-3 x ULN) in 14 (35%) patients. Serum albumin levels were reduced in 13 (51%) patients.

Patients with PC serum enzymes such as lipase, γ-GT and AP activities were significantly elevated in 14 (93%) patients. ALT and AST activities were significantly increased in 13 (86%), moderately elevated (6-8 x ULN) in 1 (10%) patients. Serum albumin levels were reduced in 12 (80%) patients. These data are summarized in Table 1. The tumor marker CA 19-9 was elevated in 15 (38%) patients with a tumor in the head of the pancreas that was confirmed by computer tomography.

The comparison of the biochemical data revealed a direct association between amylase and lipase elevations with the levels of TNF-α and INF-γ, and of the γ-GT and AP elevation and the INF-γ level in patients with CP and pancreas carcinoma (PC).

With respect to the blood levels of pro-and anti-inflammatory cytokines in the 25 patients with CP, there was a significant elevation of IL-2R and IL-6 in patients as compared to healthy controls. The mean values of TNF-α was increased in 16 (65%) and of INF-γ in 20 (82%) patients, respectively, the elevation of inflammatory cytokines closely correlated with the relevant clinical and laboratory parameters of CP of our patients (Table 1). 10 (42%) patients had drastically improved IL-6 and IL-2R levels and 14 (58%) showed only a moderate increase.
Patients with PC had a significant elevation of IL-2R and IL-6 compared to healthy controls in 14 (93%), moderately elevated (4-6 x ULN) in 1 (10%) patients. The mean values of TNF-α was increased in 14 (93%) and of INF-γ in 13 (86%) patients, respectively, the elevation of inflammatory cytokines closely correlated with the relevant clinical and laboratory parameters of PC of our patients significantly elevated in 14 (93%) patients (Table 2).

Ultrasonography and computer tomography showed a statistically significant direct association between the level of TNF-α and the degree of pancreatic necrosis, and of INF-γ and the inflammation of the pancreatic tissue. A close correlation was further found between the serum levels of IL-2R and IL-6 and the degree of the oncogenic impairment of pancreas.

### Table 1. Standard laboratory analyses of serum in patients with CP and PC

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipase</th>
<th>AP</th>
<th>γ-GT</th>
<th>ALT</th>
<th>AST</th>
<th>LDH</th>
<th>CRP</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with CP (n=25)</td>
<td>346.6±15.6*</td>
<td>454.2±15.9*</td>
<td>877.5±30 .7*</td>
<td>159.4±6.7*</td>
<td>159.7±7.2*</td>
<td>364.1±13.8*</td>
<td>66.3±2.4*</td>
<td>112.4±4.5</td>
</tr>
<tr>
<td>Patients with Pancreas carcinoma (n=15)</td>
<td>450.5±20.5*</td>
<td>506.4±8.5*</td>
<td>941.6±24.8*</td>
<td>263.5±7.2*</td>
<td>164.4±4.1*</td>
<td>390.6±34.1*</td>
<td>82.4±6.4*</td>
<td>181.2±6.2*</td>
</tr>
<tr>
<td>Controls (n=25)</td>
<td>60±2.7</td>
<td>130±3.5</td>
<td>60±2.1</td>
<td>50±2.1</td>
<td>50±2.2</td>
<td>22.5±8.5</td>
<td>5±1.8</td>
<td>106±4.2</td>
</tr>
</tbody>
</table>

*p<0.001 relative to control

### Table 2. Serum levels of inflammatory cytokines (pg/ml) in patients with CP and PC

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α</th>
<th>INF-γ</th>
<th>IL-2R</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with CP (n=25)</td>
<td>158.7 ± 19.6*</td>
<td>823.3 ± 97.5*</td>
<td>1622.7 ± 23.4*</td>
<td>138.5 ± 2.6*</td>
</tr>
<tr>
<td>Patients with PC (n=15)</td>
<td>290.8 ± 21.4*</td>
<td>952.4 ± 85.3*</td>
<td>2212.56 ± 36.5*</td>
<td>245.6 ± 6.6*</td>
</tr>
<tr>
<td>Controls (n=25)</td>
<td>43.5 ± 6.1</td>
<td>38.9 ± 5.5</td>
<td>390 ± 15.5</td>
<td>28.5 ± 1.1</td>
</tr>
</tbody>
</table>

*p<0.001 relative to control
DISCUSSION

In the majority of our patients with CP the clinical symptoms were non-specific with laboratory findings typical for CP (elevation of serum lipase and amylase). The most common clinical and laboratory findings were male gender, overweight, an enlarged head of the pancreas and elevated serum levels of glucose, lipase, and amylase. The computer tomography and ultrasonography of the pancreas was characterized by an enlarged pancreatic head with compression of the bile duct, an elevated tumor marker CA 19.9 in serums, as well as interlobular inflammatory infiltration, the presence of pseudo cysts and fibrosis.

The normal function of the immune system is based on the balanced regulatory cytokine production. In patients with CP the serum levels of IL-2R, IL-6, TNF-α, and INF-γ were elevated compared to the control group. High levels of TNF-α and INF-γ in serum of patients with CP indicate a shift towards the production of pro-inflammatory cytokines. Possible inducers of these cytokines may be endotoxins, reactive oxygen species and lipid peroxidation products. These promote the secretion of cytokines by mononuclear phagocytes through the activation the nuclear transcription factors (NF-κB).[3] TNF-α interferes with function of mitochondrial cytochrome oxidase, and stimulates the activity of succinate dehydrogenase, transferring electrons to coenzyme Q. Coenzyme Q, in turn, reacts with oxygen to form superoxide anion radicals.

One of the important mechanisms contributing to the development of CP is oxidative stress.[4,6] The presence of oxidized fat in the pancreas is evident an obvious trigger of the lipid peroxidation cascade. Fat accumulation in the pancreatic cells is possible due to increased free fatty acids (FFA) entering the pancreas, excess of FFA synthesis, a decreased FFA β-oxidation in mitochondria and a reduced synthesis or secretion of very low density lipoproteins. FFA enter the pancreas from the small intestine or adipose tissue, or are synthesized directly in the gland. During this process reactive oxygen species are formed which induce lipid peroxidation.[4,5]

Drugs inducing CP, such as NSAID, progestin’s, amiodarone and others, inhibit oxidation in the mitochondria and interfere with the electron transfer in the respiratory chain. This process promotes lipid peroxidation reactions, which result in impairment of the pancreas and CP development. On-drug induced CP requires an additional source of oxidative stress, which can initiate lipid peroxidation. It is known that the endotoxins induce cytokines, including TNF-α, IL-6, IL-2 and others and are involved in the pathogenesis particularly of alcoholic CP.[1,3]

In recent years, much attention was paid to the pathogenesis of CP, in particular to factors that promote the transition of CP to the pancreas carcinoma. The presence of oxidized fat is considered as one of the factors initiating lipid peroxidation processes. Lipid peroxidation products (aldehydes) can damage the pancreocyte membrane and activate pancreatic macrophages resulting in inflammation and fibrosis.[2,6] However, many patients never progress to the stage of inflammatory-necrotic changes and fibrosis that the development of CP requires other factors, resulting in the formation of reactive oxygen species. One of these factors is believed to be an increased production of cytokines by immune competent cells.

Thus, a vicious circle develops through the action of reactive oxygen species and pro-inflammatory cytokines. In addition to the above factors, insulin also stimulates the synthesis of TNF-α through the expression of TNF-receptors in adipose and muscle tissues.[4,7] Pro-inflammatory cytokines, including TNF-α, may participate in the mechanism of insulin resistance. How TNF-α is involved in this process is not completely understood, but insulin receptors (IRS-1) are important targets for TNF-α.[3,7]

TNF-α inhibits the synthesis of lipoprotein lipase, which degrades stored fat resulting in loss of body weight. Increased mobilization of the FFA in fat depots through the action of TNF-α contributes to the accumulation of fat in the pancreatic tissue. Thus, there is a correlation between pro-inflammatory cytokines, lipid metabolism and pancreatic steatosis. This process is mediated by activated cytotoxic T lymphocytes (CTL), natural killers cells (NK cells) via a secretary and a non-secretory pathway. Activated CTL and NK-cells are the main producers of TNF-α and INF-γ, and they secrete perforating and grages involved in the apoptosis.[1,2]

It is now generally accepted that chronic pancreatic injury and fibrosis may result from repeated episodes of acute pancreatic necro-inflammation (the necrosis-fibrosis sequence). Recent studies suggest that pancreatic stellate cells (PSCs), when activated, may play an important role in the development of pancreatic fibrosis. Factors that may influence PSCs activation during pancreatic necrosis inflammation include cytokines known to be important in the pathogenesis of acute pancreatitis, such as TNF-α and IL-6.[1,8]

CONCLUSION

The results of our study indicate that cytokines are involved in the pathogenesis of CP. This study has demonstrated that PSCs have the capacity to respond to cytokines known to be up regulated during pancreatitis. Persistent activation of PSCs by cytokines during acute pancreatitis may be a factor involved in the progression from acute pancreatitis to chronic pancreatic injury and fibrosis. The inflammatory multiple cell types within the tumor microenvironment often express IL-6. IL-6 produced in the bone marrow microenvironment to bone metastasis and this cytokine has a strong pro-tumorigenic activity due to its multiple effects on bone metabolism, tumor cell proliferation and survival, angiogenesis, and inflammation. Supporting the role of IL-6 in human cancer is the observation of elevated serum levels of IL-6 and sIL-6R in patients with bone metastasis and their association with a poor clinical outcome. Thus, IL-6 emerges...
as a key player at all stages of the pathogenesis of pancreatic diseases, including carcinogenesis, and a potential molecular therapeutic target.

What this study adds:
1. What is known about this subject?
   Serum levels of IL-6 are regarded as a reliable clinical indicator the severity of acute pancreatitis.

2. What new information is offered in this study?
   IL-6 emerges as a key player at all stages of the pathogenesis of pancreatic diseases, including carcinogenesis, and a potential molecular therapeutic target.

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REFERENCES

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