The essence of cytokines from experimental acute pancreatitis through organ preserving pancreatic head resection

Ph. D. THESIS

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CONTENTS

ABBREVATION

AP	-	Acute pancreatitis
ARDS	-	acute respiratory distress syndrome
CCK-8	-	cholecystokinin octapeptide
CP	-	chronic pancreatitis
CRP	-	C-reactive protein
DPPHR	-	duodenum-preserving pancreatic head resection
ERCP	-	endoscopic retrograde cholangio-pancreatography
IDDM	-	insulin-dependent diabetes mellitus
IDDM	-	insulin-dependent diabetes mellitus
IL-6	-	interleukin-6
PBDL	-	panceratico-biliary duct ligation
PPPD	-	pylorus-preserving pancreaticoduodenectomy
Qol	-	Quality of life
sc.	-	subcutaneously
SIRS	-	systemic inflammatory response syndrome
TGF-β	-	Transforming growth factor-beta
TNF-α	-	tumor necrosis factor alpha

List of full papers cited in the Thesis

I.   Takács, T., **Farkas, Gy. Jr.,** Czakó, L., Jármay, K., Mándi, Y. and Lonovics, J.: Time-course changes in serum cytokine levels in two experimental acute pancreatitis models in rats. *Res. Exp. Med.* <u>196</u>: 153-161 (1996). (IF:0.69)

II. **Farkas G Jr,** Takács T, Mándi Y, Balogh A. TGF-beta 1 and IL-6--new aspects in pancreas regeneration? *Acta Chir Hung.* 1997;36 (1-4):89-91.

III. Farkas G, Leindler L, Daróczi M, **Farkas G Jr.** Organ-preserving pancreatic head resection in chronic pancreatitis. *Br J Surg.* 2003 Jan; 90 (1): 29-32. (IF:3.772)

IV. **Farkas G Jr**, Hofner P, Balog A, Takács T, Szabolcs A, Farkas G, Mándi Y. Relevance of transforming growth factor-beta1, interleukin-8, and tumor necrosis factor-alpha polymorphisms in patients with chronic pancreatitis. *Eur Cytokine Netw.* 2007 Mar; 18 (1): 31-7. Epub 2007 Mar 30. (IF:1.5)

V. Farkas G, Leindler L, Daróczi M, **Farkas G Jr.** Long-term follow-up after organ-preserving pancreatic head resection in patients with chronic pancreatitis. *J Gastrointest Surg.* 2008 Feb;12(2):308-12. (IF:2.064)

List of full papers and abstracts related to the subject of the Thesis

Full papers

1. Takács, T., Czakó, L., Jármay, K., **Farkas, Gy. Jr.,** Mándi, Y. and Lonovics, J.: Serum cytokine level changes in L-arginine-induced acute pancreatitis in rats. *Acta Physiol. Hung.* <u>82</u>: 147-156 (1996).

2. Farkas G, Leindler L, **Farkas G Jr.** Safe closure technique for distal pancreatic resection. *Langenbecks Arch Surg.* 2005 Feb; 390 (1): 29-31. Epub 2004 Aug 28. (IF:1.245)

3. Farkas G, Leindler L, Daróczi M, **Farkas G Jr.** Prospective randomized comparison of organ-preserving pancreatic head resection with pylorus-preserving pancreaticoduodenectomy. *Langenbecks Arch Surg.* 2006 Aug; 391 (4): 338-42. (IF:1.245)

4. **Farkas, Gy. Jr.,** Takács, T., Mándi, Y. and Lonovics, J.: TGF-ß1 and IL-6: new aspects in pancreas regeneration? In: <u>*2nd World Congress International Hepato Pancreato Biliary Association (IHPBA)*</u>, (eds., A. Cavallari, A. Mazziotti and A. Principe) pp. 1275-1278, Monduzzi Editore Bologna (1996).

Abstracts

1. Takács, T., **Farkas, Gy. Jr**, Czakó, L., Mándi, Y. and Lonovics, J.: Time-course changes in serum cytokine levels in two experimental acute pancreatitis models in rats. *Digestion* 5: 340 (1994). (IF:1.885)

2. Takács, T., Czakó, L., **Farkas, Gy.Jr**., Hegyi, P., Jármai, K., Mándi, Y. and Lonovics, J.: Serum cytokine level changes in L-arginine-induced acute pancreatitis in rats. *Gastroenterology* 108: 394 (1995). (IF:9.329)

3. **Farkas, Gy.Jr**., Takács, T., Hegyi, P., Czakó, L., Mándi, Y. and Lonovics, J.: Serum cytokine level changes in two experimental acute pancreatitis models in rats. *Z. Gastroenterol*. 33: 286 (1995). (IF:1.08)

4. Czakó, L., Takács, T., Hegyi, P., **Farkas, Gy.Jr.,** Jármay, K., Mándi, Y. and Lonovics, J.: Time course changes in serum cytokine level in L-arginine-induced acute pancreatitis in rats. *Z. Gastroenterol*. 33: 283 (1995) (IF:1.08)

5. Takács, T., **Farkas, Gy Jr.,** Mándy, Y., Jármai, K. and Lonovics, J.: The role of TGF-ß1 and IL-6 in pancreas regeneration. *Gut* 39 (Suppl 3): A278 (1996) (IF:4.59)

6. Takács, T., **Farkas, Gy. Jr.,** Jármay, K., Mándi, Y. and Lonovics, J.: The role of TGF-ß1 and IL-6 in pancreas regeneration in rat. *Gastroenterology* 110: A434 (1996). (IF:9.329)

7. **Farkas Gy. Jr.,** Takács T., Lonovics J. and Balogh Á.: Pancreas regeneration in the view of cytokines. *Digestion* 58: A238 (1997). (IF:1.315)

8. **Farkas Gy. Jr.,** Takács T., Mándi Y., Jármay K., Lonovics J.: The importance of transforming growth factor β1 in pancreatic repair after pancreas resection, *Gastroenterol*. 38, A 403, 2000. (IF:9.329)

1. INTRODUCTION

1.1. Acute pancreatitis

Acute pancreatitis is a relatively common disease with an annual incidence of 10-20 cases per 100,000, population in the Western world. The majority of cases are alcohol related or due to gallstones (1). Although many etiological factors are known to be involved in triggering acute pancreatitis, however, once the inflammatory process has been initiated, the ultimate outcome is relatively independent of the causative agent. Furthermore, acute pancreatitis has a wide spectrum of clinical manifestations, which ranges from a mild edematous, self-limited disease with a fair prognosis to severe necrotizing inflammation with a fatal outcome. The overall mortality rate is about 10%, but in its most severe form, which is characterized by pancreatic necrosis, 20%-30% of patients die. The cause of death in most patients does not seem to be related specifically to the pancreatic inflammation or even to the infection of the necrotic pancreas or peripancreatic tissue that may occur. Rather death is often the result of multi organ system failure (2). In fact, multiple organ failure and septic complications in acute pancreatitis do not differ from the systemic complications of other diseases such as sepsis itself, trauma, or burn, which are included in a special group of diseases, namely the systemic inflammatory response syndrome (SIRS). The symptoms in different SIRS diseases might be very similar in consequence of tremendous activation of the cytokine cascade and inflammatory reactions

Despite numerous experimental and clinical results, the real pathomechanism of acute and chronic pancreatitis has not been established in detail yet (3, 4). Over the last 15 years the understanding and management of acute pancreatitis has radically altered.

Acute experimental pancreatitis has attracted the attention of numerous researchers principally because the primary mechanism that triggers pancreatitis at the cellular level is still undetermined. Due to inaccessibility of the human pancreas during life, the essence of our knowledge about the pathophysiology of acute pancreatitis is based upon animal studies. These reveal that initial events in acute pancreatitis might occur within the acinar cells Zymogene and lysosomal granules are made fragile by substances such as alcohol and its metabolites.

In gallstone pancreatitis, these granules appear colocalized within acinar cells. Products released by the granules lead to intracellular activation of digestive enzymes, such as trypsin, and acinar cell injury.

The initial response is closely followed by a second stage consisting of immigration of leukocytes into the pancreas, due to inflammatory mechanisms. This important phenomenon is driven by cytokines, chemokines and other inflammatory mediators secreted by resident cells belonging to the innate immune system and parenchymal and mesenchymal cells

The systemic manifestations of acute pancreatitis are now believed to be owing to the local and systemic actions of specific inflammatory cytokines (5). TNF-α, the early cytokine to be released, is a principal mediator of immune responses to endotoxin and other stimuli. It can be produced in large amounts in several organs during acute severe pancreatitis and is also believed to mediate pathophysiological changes (6,7). TNF-α can mirror the clinical signs of septic shock: hyper metabolism, fever, coagulopathies, increases vascular permeability, and vasodilatation. Systemic release of TNF-α is associated with septic shock and fatal outcome. TNF-α levels are increased in patients with acute severe pancreatitis and septic shock and appear to correlate with clinical outcome. The systemic manifestations are responsible for the majority of pancreatitis-associated morbidity and mortality and are due to the actions of specific proinflammatory cytokines such as TNF-α, IL-1, IL-8 (8).

1.2. Chronic pancreatitis

In summary, CP is characterized by progressive and ultimately irreversible pancreatic injury that manifests clinically as maldigestion and diabetes. Alcohol abuse is the most common association of CP in the Western world (9). Important advances have been made in recent years with respect to our understanding of the pathogenesis of this disease, particularly related to the mechanisms responsible for the development of pancreatic fibrosis (a cardinal feature of CP) after repeated acute attacks of pancreatic necroinflammation (the necrosis-fibrosis concept) (10). The pancreatic stellate cell is now established as playing a central role in fibro genesis (11), particularly when activated either directly by toxic factors associated with pancreatitis (such as ethanol, its metabolites, or oxidant stress) or by cytokines released during pancreatic necroinflammation. Considerable research effort also has been directed toward the genetic abnormalities that may predispose to CP. Mutations of several candidate genes related to trypsinogen activation/inactivation and to CFTR function increasingly are being recognized

for their potential disease-modifier role in distinct forms of CP including alcoholic, tropical, and idiopathic pancreatitis. Treatment of uncomplicated CP is usually conservative, with the major aim being to effectively alleviate pain, maldigestion, and diabetes, and, consequently, to improve the patient's quality of life.
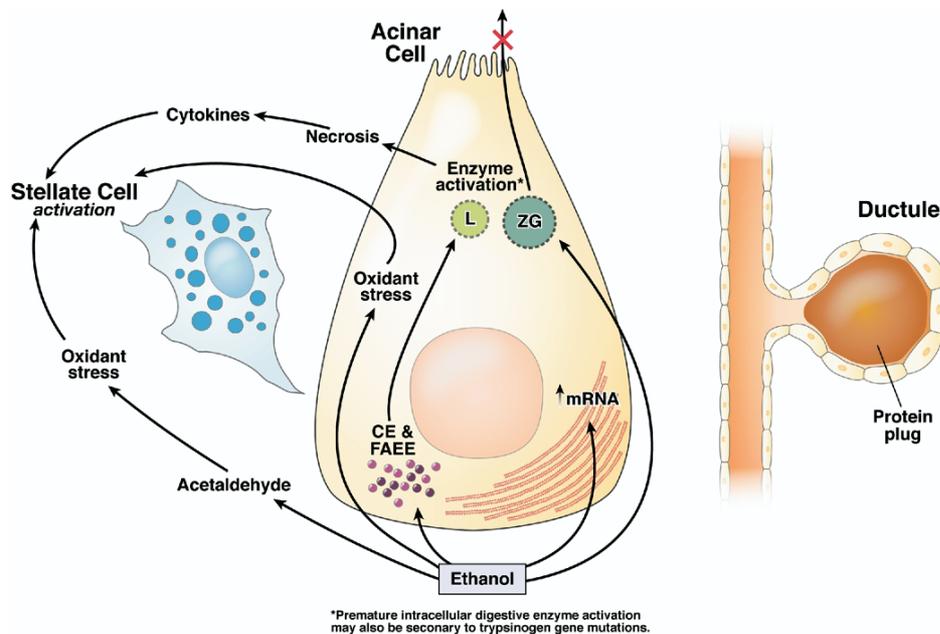
Current concept of the pathogenesis of chronic pancreatitis (12). Three major elements of the pancreas are implicated in the development of irreversible pancreatic damage. *1*: An acinar cell that is susceptible to autodigestive injury for the following reasons (depending on cause): (a) the effects of ethanol and its metabolites on sub cellular organelles including increased digestive and lysosomal enzyme content (secondary to increased synthesis [increased mRNA] and impaired secretion) and destabilization of lysosomes and zymogene granules; (b) impairment of trypsinogen activation/deactivation processes. In the presence of an appropriate trigger factor, overt acinar cell injury is initiated. *2:* A pancreatic stellate cell that
is activated by cytokines released during pancreatic necroinflammation or by direct effects of ethanol, its metabolites, and oxidant stress, leading to excessive extra cellular matrix protein production. *3:* A pancreatic ductile blocked by protein precipitation, which may further facilitate disease progression. AC, acetaldehyde; CE, cholesteryl esters; L, lysosome; ZG, zymogene granule.

1.3. Cytokines

Cytokine is a term applied to any of a rapidly growing number of small, nonstructural proteins or glycoproteins that serve as messengers between cells and are involved in such processes as cell growth and differentiation, tissue repair and remodeling, and regulation of the immune response. In acute and chronic inflammation, cytokines are instrumental in regulating the magnitude, nature, and duration of the inflammatory response (13). Cytokines also stimulate or inhibit the development of hematopietic cells.

General properties of cytokines: they are polypeptides produced in response to microbes and other antigens that mediate and regulate immune and inflammatory reactions. Cytokine secretion is a brief, self-limited event. The actions of cytokines are often pleiotropic and redundant. Cytokines often influence the synthesis and actions of other cytokines. Most cytokines act close to where they produced, either on the same cell that secretes the cytokine (autocrine action) or on a nearby cell (paracrine action). When produced in large amounts, cytokines may enter the circulation and act at a distance from the site of production (endocrine action). Cytokines initiate their actions by binding to specific membrane receptors on target cells. External signals regulate the expression of cytokine receptors and thus the responsiveness of cells to cytokines. The cellular responses to most cytokines consist of changes in gene expression of in target cells, resulting in the expression of new functions and sometimes in the proliferation of the target cells.

In clinical medicine, cytokines are important as therapeutic agents and as targets for specific antagonists in numerous immune and inflammatory diseases (14).

1.3.1 Tumor necrosis factor-alpha

Tumor necrosis factor-alpha (TNF-α) is produced during immune and host defense responses as a primary mediator of immune regulation and the inflammatory response. The major cellular source of TNF-α is activated mononuclear phagocytes, although antigen-stimulated T cells, Natural Killer (NK) cells, and mast cells can also secrete this protein(15). In macrophages, TNF-α synthesis can be induced by a wide range of stimuli for example bacterial products such as lipopolysaccharides (LPS), other cytokines: interleukin-1 (IL-1), IL-2, interferon-γ (IFN-γ), granulocyte/macrophage colony-stimulating factor (GM-CSF), TNF-α itself, complement, X-ray radiation, tumor cells, ischemia and trauma. The principal

physiologic function of TNF-α is to stimulate the recruitment of neutrophils and monocytes to sites of infection and to activate these cells to eradicate microbes. TNF-α mediates these effects by several actions on vascular endothelial cells and leukocytes (16). The biology of TNF-α is also characterized by its pathologic activities in many immune-mediated diseases. The net effects of TNF-α are influenced by a complex array of cell- and tissue-specific factors. The diverse role of TNF-α in mediating cellular responses are: the activation and induction of other cytokines such as IL-1, 6, 8, IFN-γ, transforming growth factor-beta (TGF-ß), in monocytes-macrophages and inhibition of differentiation and suppression of proliferation of these cells; increased adherence to extra cellular matrix and increasing phagocytic capacity of polymorphonuclear leukocytes; modulation of angiogenesis increasing permeability and enhanced expression of major histocompatibility complex I (MHC I) of vascular endothelial cells. TNF-α also plays an important role in the antitumor activity by inducing cell apoptosis (17, 18). The main in vivo effects are fever, anorexia, altered pituitary hormone secretion in central nervous system, and shock (19), acute respiratory distress syndrome (ARDS) (20), and capillary leakage syndrome in cardiovascular system (21). The concentration of TNF-α also influence its biological actions: at low concentrations, TNF-α acts on leukocytes and endothelium to induce acute inflammation; at moderate concentrations, TNF-α mediates the systemic effects of inflammation; at high concentrations, TNF-α causes the pathologic abnormalities of septic shock (22).

1.3.2. Interleukine-6

Interleukin-6 is a multifunctional cytokine, which is produced by lymphoid and non-lymphoid cells, such as fibroblasts, macrophages, dendritic cells, T and B lymphocytes, endothelial cells, glial cells and keratinocytes. IL-6 regulates immune responses, acute-phase reactions and haematopoiesis. The production of IL-6 is regulated by a variety of stimuli, such as IL-1, TNF, IFN-β. IL-6 induces terminal differentiation of B cells to antibody producing plasma cells. Although IL-6 is often considered as an inflammatory cytokine, most of its activities are probably associated with a negative control of inflammation as a result of its potent capacity to induce the production of acute phase proteins by the liver. IL-6 inhibits the release of IL-1, TNF and that of soluble TNF receptor.Thus IL-6 has both proinflammatory and ant-inflammatory effects (23, 24, 25).

1.3.3. Transforming growth factor-beta (TGF-β)

Virtually every cell in the body, including epithelial, endothelial, hematopietic, neuronal, and connective-tissue cells produces TGF-ß and has receptor for it. TGF-ß regulate the proliferation and differentiation of cells, embryonic development, wound healing, and angiogenesis (26). It is actually a family of closely related molecules encoded by distinct genes, commonly designated TGF-ß1, TGF-ß2, TGF-ß3. Cells of the immune system synthesize mainly TGF-ß1 (27). The principal action of TGF-ß in the immune system is to inhibit the proliferation and activation of lymphocytes and other leukocytes (28). Some regulatory T cells produce TGF-ß, and the same cells may also produce IL-10, which, like TGF-ß, has immunosuppressive activities. TGF-ß inhibit the proliferation and differentiation of T cells and the activation of macrophages (29). TGF-ß also act on other cells, such as neutrophils and endothelial cells, largely to counteract the effects of proinflammatory cytokines. TGF-ß have many diverse actions outside the immune system. TGF-ß is one of the most potent regulators of the production and deposition of extra cellular matrix (27). TGF-ß stimulates fibroblasts and other cells to produce extra cellular-matrix proteins and cell-adhesion proteins, including collagen, fibronectin, and integrins .. TGF-ß directly stimulates angiogenesis in vivo. Increases or decreases in the production of TGF-ß have been linked to numerous disease states, including atherosclerosis and fibrotic disease of the kidney, lung (31) and liver (31).

1.3.3.1 *SNP of TGF-ß1 gene*

It has been demonstrated that the production of TGF-ß varies from individual to individual and partly depends on the polymorphisms of these genes (32). The human gene encoding TGF-ß1 is located on chromosome 19q13 (33). All positions of the TGF-ß1 gene are defined relative to the first major transcription start site (position +1). The first +840 bases are a nontranslated region and codon one begins at position +841 (34). Several SNPs have been described in the TGF-ß1 gene, including a T-to-C transition at nucleotide 29 at position +869, in the region encoding the signal sequence, which results in a leucine-proline substitution at the 10th amino acid (35). It has been shown that TT homozygous genotypes are high TGF-β1 producers (36). The correlation between the TGF-ß1 gene polymorphism and the disease status has been studied in a diverse range of diseases such as heart diseases (37), acute human liver graft rejection (38), idiopathic pulmonary fibrosis (39), hypertension (40), myocardial

infarction (41), atherosclerosis (42, 43) colon, ovarian, breast cancers; diabetic nephropathy, asthma, chronic obstructive pulmonary disease (44), multiple sclerosis and osteoporosis.

1.4. Surgical treatment of CP

Approximately 20-30% of patients with chronic pancreatitis (CP) develop enlargement of the head of the pancreas in consequence of inflammatory alterations, which leads to complications such as obstruction of the pancreatic duct, common bile duct stenosis and duodenal compression. All of these are indications for surgical treatment (45). Two types of surgical procedures are currently applied in clinical practice: drainage procedures and resection operations. Drainage procedures involve a maximal preservation of the pancreatic tissue with good early results, which decrease significantly during the follow-up examination (46, 47). Resection operations comprise different types of pancreatic head resection, i.e. conventional pancreaticoduodenectomy (Whipple operation), pylorus-preserving pancreaticoduodenectomy (PPPD) (48), Beger's duodenum-preserving pancreatic head resection (DPPHR) (49) and Frey's longitudinal pancreaticojejunostomy combined with local pancreatic head excision (LPJ-LPHE) (50). Resective procedures allow effective control of the complications of CP, but these operations generally increase the exo- and endocrine insufficiencies as a consequence of the removal of an average of 35-40% of the whole gland, although duodenum-preserving resective procedures have a lower incidence of both exo- and endocrine insufficiency in comparison with PPPD or Whipple operations.

In accordance with the modern organ-preserving concept, a safe procedure has been developed for duodenum-preserving pancreatic head resection in patients with CP.

Despite the successful, modern surgical intervention in a few cases we found recurrence of the disease, and late problems. We tried to get the reason helping us our results on the basis of cytokine patomechanism in pancreatitis.

2. AIMS OF THE STUDY

As cytokines have a pivotal role in the development of acute and chronic pancreatitis, the aim of our study to investigate the role of TNF-α, IL-6 and TGF-β in both diseases. In the first part of the study experimental acute pancreatitis models were applied to explore the relevance of the cytokines in the pathomechanism of acute pancreatitis. Secondly, the role of TGF-β in the chronic pancreatitis was investigated in the clinical practice involving patients with CP. Patients with CP were stratified as to patients with mild and severe form. In the case of the severe form of CP, a new organ preserving pancreatic head resection was elaborated. Description of this new method serves the third part of the thesis; the genetic predisposion of these patients is also discussed.

The present study was designated to address the following aims:

1.: To determine the changes in serum levels of TNF-α and IL-6 with time in two experimental acute pancreatitis models in rats. To find the possibility of correlation between the degree of pancreatic tissue injury in interstitial or biliary-type acute pancreatitis and serum cytokine level was studied

2.: To investigate whether changes occur in the serum TGF-β1 levels during pancreas regeneration, and whether there is a connection between their levels and the rate of the regeneration in rats.

3.: To develop a safe and effective procedure for organ-preserving pancreatic head resection, for definitive control of the complications following the inflammatory alterations of CP.

4.: To investigate whether TGF-β1 play a role in the development of CP in human, and to find a prognostic factor for the successful treatment of CP.

3. MATERIALS AND METHODS

3.1., Experimental pancreatitis

3.1.1. Acut pancreatitis models

3.1.1.1. *Animals*

Male Wistar rats weighing 280-330 g were used in all experiments. The animals were kept at a constant room temperature of 27 $^{\circ}$C, with free access of water and a standard laboratory chow (LATI, Gödöllő, Hungary). Pancreatitis was induced either with an overdose of cholecystokinin octapeptide (CCK-8) or by ligation of the common panceratico-biliary duct (PBDL). The experiment followed the principles of laboratory animal care of the NIH.

3.1.1.2. *CCK-8- induced acute pancreatitis*

The CCK-8-induced acute pancreatitis group (n=5) received 75 ug/kg CCK-8 (sytethysed by Botond Penke, Department of Medical Chemistry, Szeged) subcutaneously (s.c.) three times at hourly intervals (51). The control group of animals (n=5) received physiological saline s.c.

3.1.1.2. *PBDL-induced acute pancreatitis*

The PBDL-induced acute pancreatitis group (n=5) were anesthetized with pentobarbital and midline laparotomy was performed. PBDL was achieved by ligating the common pancreato-biliary duct adjacent to the duodenal wall (52). The control group of animals (n=5) were subjected to a midline laparotomy (sham operation)

3.1.1.3. *Experimental protocol*

Animals were killed by abdominal aorta exsanguination 0, 2, 4, 8, 16, 24 or 48h following the last CCK-8 injection or PBDL. The pancreas was carefully removed, cleaned of fat and weighed.

3.1.1.4. *Histological examination*

A fragment of the pancreas was fixed overnight in 10% neutral formaldehyde solution for hematoxylin and eosin stunning and for histological study by light microscopy.

3.1.2. Pancreas regeneration models

3.1.2.1. *Animals*

Male Wistar rats weighing 350-450 g were used in all experiments. The animals were kept at a constant room temperature of 27 ^{o}C, with free access of water and a standard laboratory chow (LATI, Gödöllő, Hungary). Pancreas regeneration was studied during CCK-8 induced regeneration after distal pancreas resection (75%). The experiment followed the principles of laboratory animal care of the NIH.

3.1.2.2. *CCK-8-induced regeneration*

In each group the rats (n=5) were anesthetized with ether and midline laparotomy was performed. The distal part (75%) of the pancreas was resecated with preserving the spleen. CCK-8 was administered subcutaneously in a 300 ng/kg dose 3 times per day to the investigated group, while the control animals received the same amount of saline.

3.1.2.3. *Experimental protocol*

Animals were killed by abdominal aorta exsanguination 3, 7, 14, and 28 days after the first injection. The residual pancreas was carefully removed, cleaned of fat and weighed. The changes in the pancreas weight were calculated in each case to the formerly resecated weights respectively, by mathematical methods.

3.1.3 Assays

3.1.3.1. *Amylase activity*

Serum amylase activity was measured by the Phadebas test method (53).

3.1.3.2. *TNF and IL-6 assay*

TNF and IL-6 bioassay was performed applying WEHI 164 targets (54). The data are expressed as TNF units/ml, calibrated from standard rmTNF. Serum samples were also assayed after incubation for 30 min at 37°C with a polyvalent rabbit antiserum against rmTNF-α, at approximately 10^4 neutralizing units per milliliter. The cytotoxic activity of sera was completely blocked by antibody.

IL-6 was measured via its proliferative action on he IL-6 dependent mouse hybridism cell line B-9 (55). The activities were calibrated against IL-6 (Amersham International, Bucks, UK)

3.1.3.3. *Western blot analysis for TGF-β*

TGF-β was detected by an ECL-Western blot technique with the application of anti-TGF-β1 antibody. Total protein from rat pancreas samples was solubilized after ultrasonication, with subsequent processing in Laemmli buffer, and protease inhibitor cocktail(SIGMA) The individual protein samples (10 microgram/lane) were fractionated on 15 % SDS-polyacrilamyde gels. After electrophoresis, proteins were transferred to nitrocellulose blotting membrane. Membranes were praeblocked, and immunoblotting was performed with with anti-TGF-β1 antibody. A HRP conjugated anti mouse IgG was used as secondary antibody. Antigen-antibody complexes were detected via chemiluminescence reaction (ECL Amersham), followed by the exposure to X-ray film (KODAK). Densitometric analysis of the blots was performed by ImageQant Software (Amersham Bioscience)

3.1.3.4. *DNA and protein content*

DNA contents of the pancreas were determined by the procedure of Giles&Meyers, protein content by the GOA method; assayed with colorimetric method.

3.1.3.5. *TGF-β assay*

For TGF-β ELISA determination venous blood was collected from the rats into EDTA-containing tubes for collecting plasma. Blood was collected 3, 7, 14, and 28 days after the first CCK-8 injection. Centrifugation was carried out at 2000g for 10 min at 4°C. All samples were stored at -20 ^{o}C. Plasma concentration of TGF-β1 was determined by enzyme-linked

immunosorbent assay kit (R&D System Inc., Minneapolis, USA) according to the instructions of the manufacturer.

3.1.4. Statistical analysis

Results were expressed as mean ± SEM. Experiments were evaluated statistically with Student's t-test for paired or unpaired values, as appropriate. *P* values less than 0.05 were accepted as significant.

3.2., Human chronic pancreatitis

3.2.1.: The role of TGF-β1 in patients with CP

3.2.1.1. *Patients*

Our study involved 83 patients (24 females and 59 males; mean age 52.7 years, range 22-70) who underwent medical or surgical treatment for chronic pancreatitis at the Department of Internal Medicine and/or Department of Surgery of the University of Szeged between 2003 and 2006. The diagnosis of chronic pancreatitis was based on the typical history (daily alcohol intake), abdominal complaints (pain, bloating, steatorrhoea, etc.) and characteristic morphologic and/or functional alterations of the pancreas. The morphologic changes due to chronic inflammation of the pancreas (pancreatic calcification on ultrasonography /US/ and/or computed tomography /CT/, mild to moderate or marked ductal lesions during endoscopic retrograde cholangio-pancreatography- /ERCP/ examination) were assessed in each case. Pancreatic calcifications were found in 31 (37.5 %) patients on US or CT.

According to the etiology, 65 of the patients (78.6%) had a history of alcohol abuse (consumption of >50 g/day) and 12 (21.4%) patients has idiopathic CP. Exocrine pancreatic insufficiency was assessed by means of a stool elastase test (56). 47 patients with stool elastase values of less than 200 μg were considered to have pancreatic insufficiency.

The endocrine function was evaluated in nondiabetic patients by means of the oral glucose tolerance test (OGTT). 56 patients (67.9%) had impaired endocrine function (latent or manifest diabetes).

3.2.1.2. *Surgical intervention*

The indication for operation was intractable pain, loss of body weight, and obstruction of the ductal system (pancreatic duct, common bile duct or the duodenum) caused by an inflammatory enlargement of the pancreatic head. Duodenum preserving pancreatic head resection (57) was performed in 8 cases, organ preserving pancreatic head resection (58) in 15 cases, pylorus preserving pancreatic head resection in 4 cases, and Wirsungo-jejunostomy in 13 cases. Rehospitalization and reoperation was necessary in 8 cases.

The control group consisted of 75 age- and gender-matched healthy blood donors, who had no gastrointestinal or liver diseases, were selected locally from consecutive blood donors in Szeged, Hungary. The study protocol was approved by the Ethical and Science Committee of the Ministry of Health and the University of Szeged Regional and Institutional Committee of Science and Research Ethics.

All participating subjects were of Hungarian ethnic origin and resident in Hungary.

3.2.1.3. *DNA extraction*

For the examination of TGF-ß1 polymorphisms, genomic DNA purified from peripheral blood was used. Leukocyte DNA was isolated using the High Pure PCR Template Preparation Kit according to the manufacturers' instructions (Roche Diagnostic GmbH, Mannheim, Germany) and the genomic DNA was stored at -20°C until further use.

3.2.1.4. *Determination of TGF-ß1 +869 T$\rightarrow$C polymorphism*

The defined single-nucleotide polymorphism T^{29}-C in exon 1 of the human TGF-ß1 gene was determined with an amplification refractory mutation system $-$ARMS- (59) with a generic primer (sense), (5'-TCCGTGGGATACTGAGACACC-3'); and with two allele-specific antisense primers, differing from each other in only one base at the 3'-end- primer C: 5'-GCAGCGGTAGCAGCAGCG-3' and primer T: 5'-AGCAGCGGTAGCAGCAGCA-3' (Fig.2.).
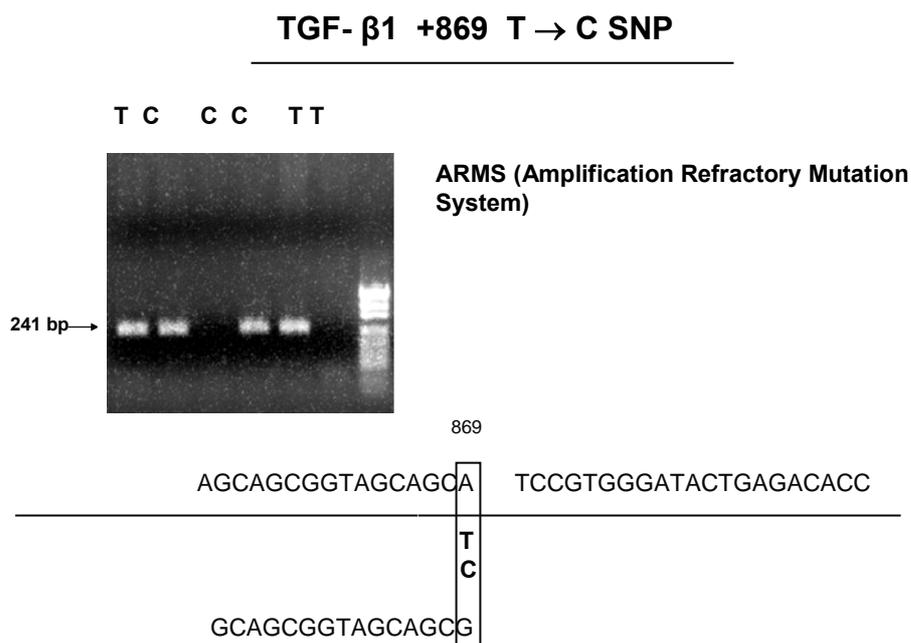
TGF- β1 +869 T → C SNP

T C   C C   T T

ARMS (Amplification Refractory Mutation System)

241 bp→

869

AGCAGCGGTAGCAGCA | TCCGTGGGATACTGAGACACC

T
C

GCAGCGGTAGCAGCG

Fig.2. Detection of TGF-β1 + 869T →C SNP by ARMS

3.2.1.5. *TGF-β1 ELISA*

Venous blood was collected from healthy blood donors and patients with chronic pancreatitis into EDTA-containing tubes for collecting plasma. Blood was collected from surgical patients before the operation. Centrifugation was carried out at 2000g for 10 min at 4°C. All samples were stored at -20 °C. Plasma concentration of TGF-β1 was determined by enzyme-linked immunosorbent assay kit (R&D System Inc., Minneapolis, USA) according to the instructions of the manufacturer.

3.2.1.6. *Statistical analysis*

Statistical analyses for comparison of allele and genotype frequencies between groups were performed by using the χ^2 test and Fisher's exact test if one cell had n < 5. The probability level of p<0.05 indicated statistical significance. The relationship between genotypes and disease severity is presented as the odds ratio (OR), with a 95% confidence interval (95% CI).The genotype frequencies for each polymorphism were tested for deviation from the Hardy-Weinberg equilibrium by means of the χ^2 test, with one degree of freedom used. The levels of TGF-β1 in the plasma were compared by means of one-way ANOVA. The

Bonferroni test was used for post hoc pair wise multiple comparisons. In all tests, an α level of p< 0.05 was taken as an indication of statistical significance. All statistical calculations were performed with the GraphPad Prism4 (GraphPad Software Inc., San Diego, CA) statistical program.

3.2.2.: New organ preserving pancreatic head resection in patients with CP

3.2.2.1. *Patients*

Since February 1999, a new surgical procedure has been performed in 135 patients (103 men and 32 women; mean age: 49.5 yrs [range 28-63]) after the development of an inflammatory tumor of the pancreatic head (median diameter 68 mm [range 46 to 129 mm], as assessed by helical CT scan). The preoperative morbidity involved frequent, sometimes severe abdominal pain, a significant loss in body weight in all patients, jaundice in 10 patients, and latent and insulin-dependent diabetes mellitus (IDDM) in 16 and 21 patients, respectively. The mean interval between the appearance of the symptoms and the surgical intervention was 7.8±2.2 yrs. The etiology was connected with chronic alcohol ingestion in 86% (117 patients), the CP was associated with biliary stone disease in 14 patients (10%), and it was unknown in 4 patients. The diagnosis was confirmed by ERCP, sonography and the CT scan. ERCP revealed that the diameter of the main pancreatic duct varied between 3 and 9 mm. In the 10 icteric patients and in 15 patients without jaundice, the common bile duct was stenotic, due to inflammatory tumor compression with prestenotic dilatation, combined with high levels of alkaline phosphatase (1035±152 U/L). The CT scan demonstrated parenchymal calcification in 72 patients; 21 patients had pseudocystic cavities, and in 4 of them a pseudocyst caused a sub acute inflammation in the pancreatic head. No patient exhibited portal hypertension.

Before the operation, prophylactic antibiotic (ceftriaxone) was used and in the early postoperative period all of the patients were treated by standard supportive treatment, consisting of total parenteral nutrition for 4 days, a proton pump antagonist (pantoprazole), suppression of TNF synthesis (pentoxifylline) and octreotide medication (60). The oral nutrition was started on postoperative day 5.

Pancreatic functions were checked by means of stool elastase determination with a sandwich ELISA method (Pancreatic Elastase1®, ScheBo Biotech, Giessen, Germany) (61). The glucose tolerance test was applied to check the endocrine function. Blood glucose levels were

measured after 0, 30, 60, 90 and 120 min by means of a glucose oxidase assay following the administration of 75 g oral glucose.

3.2.2.2. *Operative procedure*

The surgical procedure involved a wide local resection of the inflammatory tumor in the region of the pancreatic head, and decompression of the organ and the intrapancreatic segment of the common bile duct if the prepapillary duct had become stenotic. The operative procedure started with the Kocher maneuver, partial dissection of the gastrocolic ligament for mobilization, and exploration of the head of the pancreas, without division and cutting of the pancreas over the portal vein. An intraoperative frozen section was performed for all patients; none of them revealed signs of malignancy. The following step of the operative procedure was ligation of the pancreaticoduodenal artery and the veins directed to the duodenum and to the superior mesenteric vein. The enlarged pancreatic head was excised in almost its entirety, leaving behind a bridge of pancreatic tissue about 10 mm wide, while a rim of pancreas (5 to 10 mm) remained beside the duodenum and on the upper margin of the pancreatic head. This wide excision gives a possibility for drainage of the pancreatic juice from the distal pancreas and for opening of the prepapillary obstructed common bile duct in the icteric patients and in patients with a stenotic common bile duct. The prestenotic dilatated common bile duct was opened with an incision about 8-10 mm long, and the opened duct wall was sutured to the surrounding pancreatic tissue with interrupted Vicryl[®] 3/0 sutures. After careful hemostasis of the operative region, the reconstruction, with drainage of the secretion from the remaining pancreas into the intestinal tract, took place through a jejunal Roux-en-Y loop, with application of one-layer interrupted Vicryl[®] 2/0 sutures (Fig.3.) (62). There was no indication or necessity for blood transfusion during the operation. The mean operating time was 165 min (range 120 to 210 min).
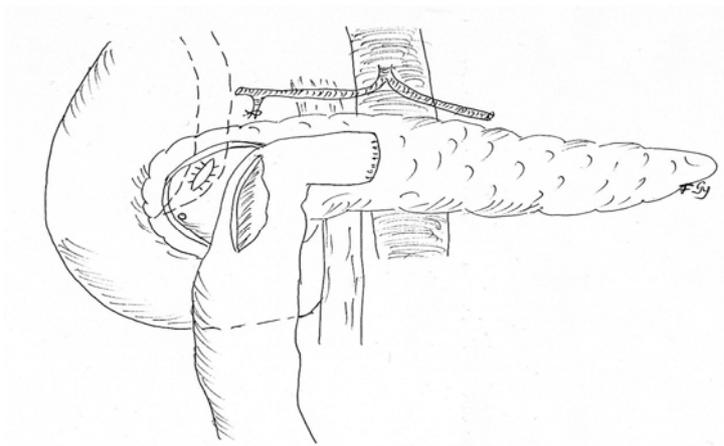
Fig. 3. The operation consists in a wide local resection of the inflammatory tumor in the region of the pancreatic head, without division and cutting of the pancreas over the portal vein. Reconstruction, with drainage of the secretion from the remaining pancreas into the intestinal tract, takes place through a jejunal Roux-en-Y loop. In icteric cases, prepapillary bile duct anastomosis is also performed with the jejunal loop.

3.2.2.3. *Quality of life*

The QoL and pain score before and after surgeries were assessed by using the European Organization for Research and Treatment of Cancer (EORTC) Quality-of-Life Questionnaire (QLQ-C30) (63). The EORTC QLQ-C30 has been re-evaluated and demonstrated to be a valid and reliable tool to measure the QoL in patients with benign disease such as CP (64). The EORTC QLQ-C30 comprises items relating to the physical status, the working ability, the emotional, cognitive and social functioning, and an overall QoL scale. Pain intensity was estimated by means of a pain scoring system including a visual analog scale, the frequency of pain attacks, the use of analgesic medication, and duration of the inability to work. The overall pain score was given by the sum of the individual values divided by 4. This questionnaire was prospectively assessed at two time points during the study: before the surgical procedure and in the follow-up period (a mean of 4.1 years) after the operation.

3.2.2.4. *Statistical analysis*

Statistical significance was estimated by using Student's t test or the Wilcoxon rank test, as appropriate. The level of significance was set at $P<0.05$. The results on the parametric data are expressed as means $\pm$ standard deviation. Nonparametric data are expressed as medians.

4. RESULTS

4.1. Experimental acute pancreatitis

4.1.1: CCK-8-induced pancreatitis

The ratio pw/bw was significantly increased after the induction of acute pancreatitis and reached its maximum level 4 h after the last CCK-8 injection (8.19±1.13 vs. 4.72±0.64 mg/g). After 4 h, pw/bw decreased continuously, and it had normalized by 24 h.

The serum amylase activity rose steadily up to 4 h after the last CCK-8 injection ($69.4\pm12.8\text{x}10^3$ vs.$3.12\pm0.28\text{x}10^3$ U/ml). It had reached the control level at 16 h.

In the CCK-8-induced pancreatitis, the serum IL-6 level had begun to increase at 2 h, but the peak level (123.3±5.7 pg/ml) was reached at 4 h. Thereafter it decreased, but it was still higher than the control (37.5±15 pg/ml). (Fig.4.) Detectable TNF was found in CCK-8-induced pancreatitis only at 2 h (25.5±5 U/ml).
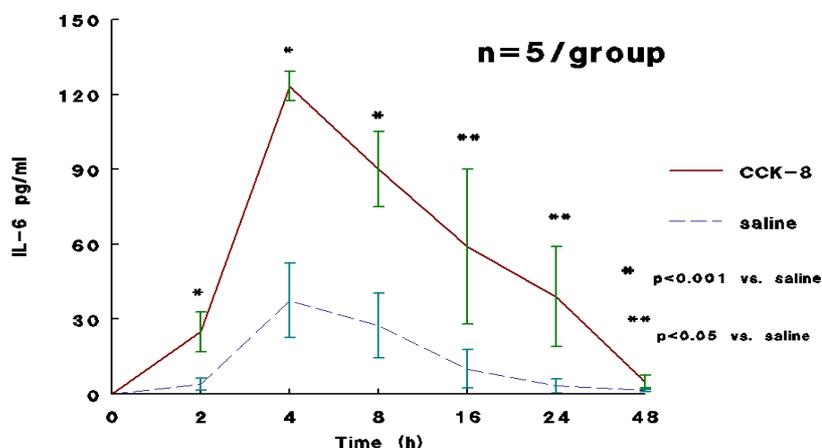

Fig.4. Changes in serum IL-6 levels with time in CCK-8-induced acute pancreatitis

Macroscopic study of the pancreata in the CCK-8-treated group revealed interstitial edema. Microscopic examination demonstrated a moderate diffuse parenchymal degeneration, including vacuolation and focal necrosis (dark cells) within the acinar cells. These morphological changes were most evident at 8h and gradually decreased thereafter. The interstitium remained virtually unchanged.

4.1.2. PBDL-induced acute pancreatitis

In PBDL-induced pancreatitis, the increase in pw/bw was fairly continuous and it reached its maximum level lat 48 h (8.8±1.4 vs. 5.3±0.8 mg/g). The serum amylase activity peaked 2 h after PBDL (43.2±13x10^3 U/ml), and then decreased continuously.

The maximum elevation in serum IL-6 level in the PBDL model was observed at 16 h (3800±447 pg/ml). The laparotomy itself (sham operation) also resulted in a moderate elevation of the serum IL-6. (Fig.5.)
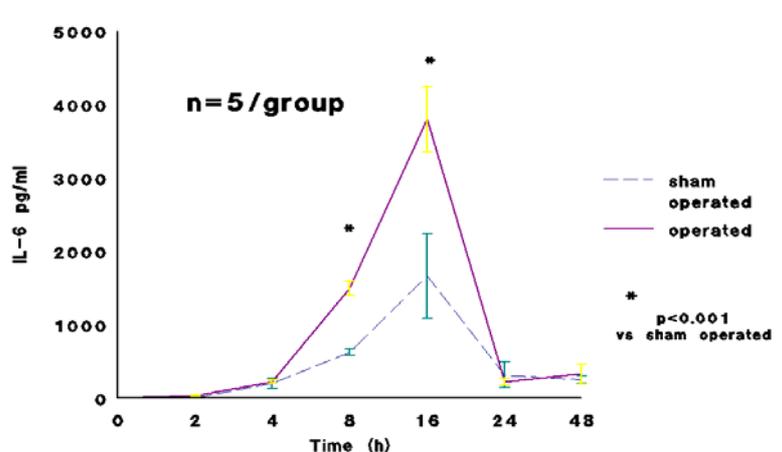

FIG.5. Serum IL-6 levels changes in PBDL-induced acute pancreatitis.

The serum TNF level was elevated 8,16 and 24 h after PBDL (Table 1.)

Time (h)	CCK-8-induced		PBDL-induced	
	Control	CCK-8	Control[a]	Operated
0	<5	<5	<5	<5
2	<5	25±5	<5	<5
4	<5	<5	<5	<5
8	<5	<5	<5	125±25
16	<5	<5	50±15	300±85
24	<5	<5	<5	75±15
48	<5	<5	<5	<5

Table 1. Serum TNF levels (U/ml) in acute pancreatitis models in rats

Values are mean ±SEM for groups of five rats   [a] Sham operated

On visual inspection, the pancreata were observed to be enlarged and edematous, with occasional hemorrhages. Histologically, increasing edema and infiltration by neutrophilic leukocytes and mononuclear phagocytes were seen in the interstitium of the pancreas 2 h after ligation. The acinar lumen was dilated and filled with secretion, and the acinar cells were partly degranulated. In contrast, the ducts and blood vessels remained unaltered. These changes progressed during the experiment. By 8 and 16 h after the ligation, cytoplasmic vascuolation of the acinar cells was seen and the acinar cells appeared to be necrotic. By 24 and 48 h, areas of intraparenchymal hemorrhage were observed.

4.1.3. CCK-8 induced pancreas regeneration

In the *pancreas regeneration model the wet weights* of the residual pancreas increased in both groups up to day 3. Subsequently, the weights decreased in the controls, but increased continuously in the CCK-8-treated group. There was a significant difference on day 14, and on day 28 the pancreas weight was almost doubled in the CCK-8-group, whereas in the controls it has decreased to normal level.

The protein content reached its highest level on day 28 in the CCK-8-treated group (32.435±7.88 mg/pancreas). A significantly higher level of IL-6 was measured on days 7 vs. the control (250±70 v. 50±30 pg/ml). It later decreased, but remained above the control level.

 The DNA content of the pancreas was continuously higher in the treated than in the control group. It reached the maximum level on day 28, with significant difference (1850±350 vs. 780±240 γ/pancreas).

Significantly different *TGF-β1 levels* were measured on days 7 and 14 (290±40 vs. 155±60 and 295±8 vs. 155±55 ng/ml, respectively). There was no difference between the TGF-B1 levels in the two groups on day 28. (Fig.6.)
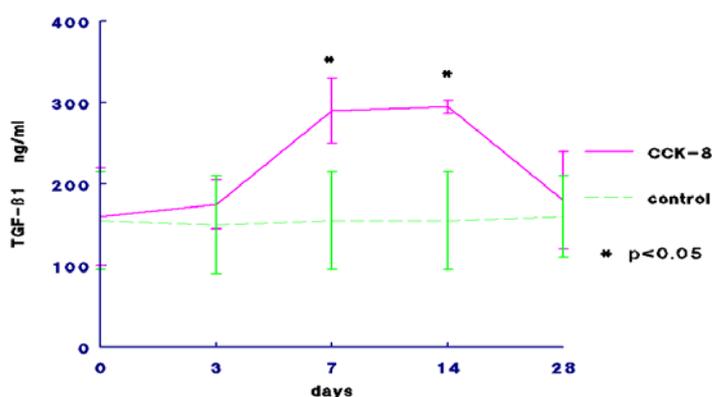
Fig.6. TGF-β1 levels changes in CCK-8-induced pancreas regeneration

Western blotting was applied for the immunodetection of TGF-β in protein lysates of pancreas samples, following CCK induction. Figure 7 reveals a representative result of time-course investigation, showing, that TGF-β expression in the rat pancreas increased from.3 days following CCK treatment up to the 7th day, thereafter the TGF-β level returned to the basal level.,These data underline the role of TGF-β in the regeneration in experimental pancreatitis.
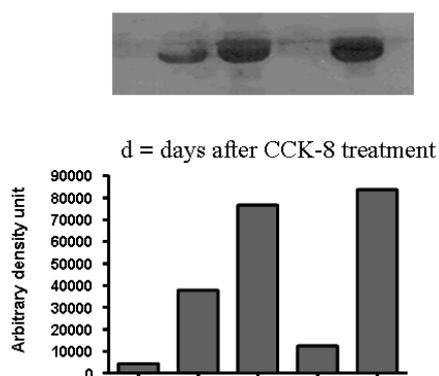

Fig.7. TGFβ-1 expression in rat pancreatic tissue

 No significant changes were observed in the *amylase levels*; they remained at a normal level (5.3±0.5 U/ml). This indicates that he increase in the pancreas weight was not caused by pancreatitis.

4.2.: Human chronic pancreatitis

4.2.1. Experimental results

4.2.1.1. *TGF-ß1 +869 T→C polymorphism in patients with CP*

The genotypic distribution of the +869 T→C polymorphism of the TGF-β1 gene is shown in Table 2. The distribution of the TGF-β1 genotypes was in accordance with the Hardy - Weinberg equilibrium in the control population (χ^2 = 2.95; p = 0.2676), but not in the patient group with chronic pancreatitis (χ^2 = 5.215, p = 0.022).

	TT	TC	CC	
operated	25/40 (62%)	10/40 (25%)	5/40 (13 %)	*p=0.001**
	p= 0.0007 vs.contr [†]			
non operated	17/43 (39.5%)	17/43 (39.5%)	9/43 (20%)	
	p=0.223 vs.contr ns [†]			
	p=0.0486 vs. operated [†]			
Total	42/83 (50%)	27/83 (33%)	14/83 (17%)	*p=0.009**
	p=0.005 vs.contr [†]			
Control	21/75 (28%)	30/75 (40%)	24/75 (32.%)	

Table.2. TGF-β1 +869 genotype distribution in patients with chronic pancreatitis and in control subjects

ns=non significant   * chi - square test vs.controls   [†] Fisher test

No correlation was found between the genotypes and histological varieties of chronic pancreatitis

There was a significant difference in genotypic distribution between the chronic pancreatic patients overall and the healthy controls (p = 0.009, χ^2 = 9.409). When the patients were stratified according the progression of the disease - i.e. medical treatment or surgical treatment – a significant difference was observed only between the controls and the operated

patients (p = 0.0012) and not between the controls and the patients with only medical treatment. To elucidate the reason for this difference, we compared the numbers of TT homozygotes among the patients and the healthy controls. The frequency of TT homozygote's (high TGF-β1-producing phenotype) were significantly higher in the patient group overall (50%) than in the controls (28%) (p=0.005; OR =2.634; 95% CI = 1.358-5.111). There was an even higher frequency of the TT genotype among patients with surgical intervention as compared with the controls , 62 % vs. 28 %, p = 0.0007, OR = 4.018, 95 % CI = 1.796 - 8.987 There was also a significant difference between the operated patients, and those treated medically (p = 0.0486, OR=2.549, 95%CI=1.052-6.178). Though the frequency of the TT genotype was still higher among the patients in the medically treated (non - operated) group (39.5%) than in the controls, the difference was not statistically significant. No further significant differences were observed as regards the SNP-s when the patients were stratified according to the presence or absence of calcification. The frequency of the T/C genotype was significantly higher in both groups of patients than in the controls (58 %, and 58% vs. 40%)

4.2.1.2. *TGF-β1 plasma levels*

Plasma levels of TGF-β1 were higher in the patients overall than in controls (3.98 ±1.26 ng/ml vs. 2.1 ± 0.85 ng/ml), and higher in the patients with TT genotype than in those with the CT and the CC genotypes (5.2 ± 1.7 ng/ml vs. 3.8 ± 1.1 ng/ml and vs. 1.5 ± 0.5ng/ml respectively; p< 0.001 ANOVA). A similar tendency was observed in the control group; the subjects with TT genotype exerted the highest plasma TGF-β1 levels (2.8 ± 0.9 ng/ml) (Table 3). However, the plasma TGF-β1 concentrations differed significantly between the patients and the controls, both in the TT homozygote groups and in the TC heterozygote groups. (p < 0.001 statistically significant are according the Bonferroni post test.) No significant difference was observed between the"low - level" TGF-β1 concentrations when the patients and controls were CC homozygote.

Those patients who had TT genotype with high serum levels of TGF-β1 were rehospitalized or underwent further operations. (Fig.8.)

TGF-β1 plasma levels ng/ml

Genotype	Control subjects	Patients with CP
TT	2.8 ± 0.9 • *n = 21*	5.2 ± 1.7 * *n = 42*
TC	2.2 ± 0.9 *n = 30*	3.8 ± 1.1 *n = 27*
CC	1.2 ± 0.7 *n =24*	1.5 ± 0.5 *n =14*

Table3. TGF-β-1 plasma levels in patients with chronic pancreatitis (CP) and in control subjects. Data are means ± SD.

* $p < 0.01$ ANOVA test on patients
• $p < 0.05$ ANOVA test on controls

Bonferoni post-test analysis revealed significantly differences between all the TGF β-1 values, apart from between patients and controls with the CC genotype, and between the controls with the TC and CC genotype.
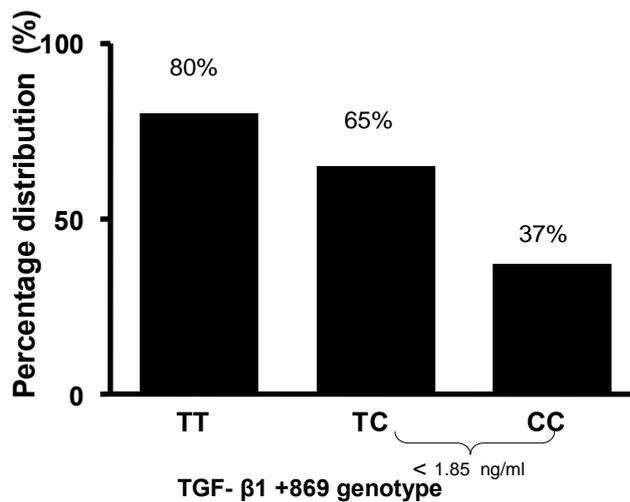

Fig.8. Recurrence (%) in chronic pancreatitis

4.2.2. Surgical results

In 135 patients, the OPPHR procedure was performed after the development of an inflammatory tumor of the pancreatic head. In the postoperative period, only one reoperation was required in consequence of anastomosis bleeding, another case was treated conservatively, and one patient had pneumonia, but no septic complication, anastomosis insufficiency or other problems; the morbidity was therefore 2.9%. There was no mortality in the postoperative period. In the 25 icteric and common bile duct stenotic patients, the liver functions normalized (serum bilirubin <22 μmol/L, and alkaline phosphates 332±92 U/L; as compared with the preoperative data, the reduction was significant [$P<0.05$]) following the operation. The duration of hospitalization ranged between 7 and 12 days, with a median of 8.5 days. The histological examinations confirmed fibrosis and calcification in 63 and 72 patients, respectively.

The mean follow-up period was 4.1 years (range 0.5 to 7.0). Five patients were lost to follow-up (3.7%). Complete follow-up data on 130 patients were included in the evaluation; the follow-up rate was therefore 96.3%. 116 patients became complaint-free (89%), 14 patients had moderate symptoms and the body weight increased by a mean of 11.3 kg (range 4-28) ($P<0.05$). Within 2 years following operations, 5 patients were reoperated: a bilio-digestive bypass was performed in consequence of developed bile duct stenosis. In the follow-up period, a further 6 patients were admitted to the clinic with an acute episode of pancreatitis; all of them were treated conservatively. Readmission was therefore necessary 11 of the 130 patients (8.4%). The late mortality was 3.7% (5 patients); the reason was cardiovascular failure or an accident, 4 and 1 patient respectively. The stool elastase level increased slightly, but not significantly (from 124.3±33 to 132±39 μg/g; NS). The preoperative and postoperative endocrine functions remained in almost the same stage: 95 patients were normoglycemic, 6 had latent DM and 20 had IDDM, but 9 patients with latent DM became IDDM (6.6%).

Both before the operation and during the follow-up, the patients were asked to complete the QoL questionnaire (EORTC QLQ-C30). A full answer was obtained from 105 patients (78%). The questionnaire was compared at two time points: *(1)* before the operation and *(2)* at a mean follow-up of 4.1 years (0.5-7) after the operation. The median pain score decreased by 91% ($P<0.001$) after surgery. No patient suffered a frequent pain attack and only 10% of the patients mentioned moderate pain occasionally without any pain killer medication (Table 4).

During the follow-up, the median global QoL improved by 100%. Apart from the cognitive functioning, the physical status, working ability, emotional and social functioning all improved significantly ($P<0.05$). The results of the symptom scales are summarized in Table 5.

Table4. Preoperative and follow-up pain scores (n=105)

Criterion	Preoperative score (Median [range])	Follow-up score (Median [range])	
Pain visual analogue scale	82 (55-100)	10 (0-15)	
Frequency of pain attack	75 (50-100)	12.5 (0-15)	
Pain medication	20 (20-100)	0 (0-100)	
Inability to work	75 (75-100)	0 (0-100)	
Pain score	63 (50-100)	5.6 (0-37.5)	($P<0.001$)*

*Preoperative values were compared with follow-up values by the Wilcoxon rank sum test

Table5. Preoperative and follow-up functioning scale scores (n=105)

Functioning scale	Preoperative score (Median [range])	Follow-up score (Median [range])
Physical status	60 (20-100)	70 (20-100) ($P<0.05$)*
Working ability	50 (0-100)	70 (0-100) ($P<0.05$)*
Cognitive	50 (40-80)	66.7 (40-100) NS*
Emotional	25 (0-75)	66.7 (40-100) ($P<0.05$)*
Social	16.7 (0-66.7)	66.7 (0-100) ($P<0.05$)*
Overall quality of life	28.5 (14.3-57.1)	57.7 (33.3-100) ($P<0.05$)*

*Preoperative values were compared with follow-up values by the Wilcoxon rank sum test
 NS: not significant

5. DISCUSSION

5.1., Both the administration of high dose of CCK-8 and ligation of the pancreato-biliary duct are suitable methods for the induction of acute pancreatitis, but biochemical and morphological examination revealed differences in the severity of changes. The over dosage of CCK-8 resulted in a mild edematous (interstitial) pancreatitis, while PBDL caused a more severe necro-hemorrhagic pancreatitis (65, 66, 67).

The serum IL-6 level began to rise after the induction of acute pancreatitis and its peak preceded the most severe morphological alterations and the maximum amylase concentration in both models. The maximum IL-6 level was 30 times higher in the PBDL model than in the interstitial pancreatitis model. It is noteworthy that the sham operation itself caused a transient elevation of the serum IL-6 level. In human, an increased serum IL-6 concentration has been observed after abdominal surgery. This might be the explanation for the increased cytokine levels in the sham-operated control animals.

Only a moderate TNF-α elevation was detected in CCK-8 induced acute pancreatitis, whereas its level was increased about 10-fold in the PBDL group. The induction of TNF occupies a key position in the elevation of IL-6, as it reflects the hierarchy in the induction of cytokine cascade (68). It is very likely that in our experiments the elevation of IL-6 was always the consequence of TNF induction.

In the PBDL model particularly high IL-6 levels were observed and, concomitantly, significantly higher TNF concentrations could be detected. It is likely, that more potent the agent inducing TNF-α, the higher the IL-6 production in the experimental animals. Moreover, the results lend support to the hypothesis of leukocyte activation as a key event in severe acute pancreatitis. The determination of C-reactive protein (CRP) might be useful as well in the diagnosis of pancreatitis and in assessment of severity of the disease (69). However, Gross et al (8).demonstrated that peak serum level of IL-6 precedes the maximum concentration of CRP by 1-2 days, in accordance with the observation and to IL-6 production. We regard the elevation of IL-6 serum levels in our experiments as an early marker of general leukocyte activation.

We could demonstrate correlations between the laboratory and morphological parameters of pancreatitis and the serum IL-6 levels in both experimental acute pancreatitis models.

5.2., From the lessons we learned about the roles of cytokines in acute pancreatitis, we checked furthermore whether cytokines have some regulatory functions in the pathomechanism of chronic pancreatitis.

Earlier reports suggested that TGF-β isoforms may act by both autocrine and paracrine mechanisms in the pancreas. In CP and in pancreatic cancer, TGF-β1 involvement has been proved. Our study revealed a transient increase in TGF-β1 levels in the CCK-8 treated group. The significant changes in the pancreas weight and the protein and DNA contents indicated pancreas regeneration and some degree of fibrosis. The TGF-β1 level remained high until day 14, and then decreased. Additionally the pancreas tropism induced by CCK-8 could be detected in increasing level up to the end of the first month. After cerulein-induced pancreatitis, TGF-β1 was found to be involved in the regulation of extra cellular matrix regeneration (70). Our findings revealed that regular low-dose CCK-8 injections resulted in pancreas regeneration following 75% distal resection. This was indicated by increases in pancreas weight, and in the DNA and protein contents of the pancreas. Significantly elevated serum TGF-β1 levels were also detected. These data show that cytokines can take part and modulate the development of the pancreas, and suggest roles for TGF-β1 in regulating the in vivo regeneration of the pancreas.

5.3., Enlargement of the pancreatic head due to chronic inflammation causes permanent pain, obstruction of the pancreatic duct alone or together with the common bile duct, and duodenal compression. With these complications, surgical treatment is generally indicated. The aims of surgical therapy, therefore, are not only to eliminate pain, to manage the CP-associated complications of the adjacent organs, and possibly to preserve the endocrine and exocrine functions, but also (more importantly) to improve the patients' overall QoL and physical status, and also to provide for their social and occupational rehabilitation (71). The objective outcome assessment of surgical treatment was made with the EORTC QLQ-C30, which has previously been demonstrated to be a valid and reliable with tooling which to measure the QoL in patients suffering from benign diseases such as CP (64, 72).

In the past, classical Whipple's pancreatoduodenectomy (PD) and PPPD were applied as standard surgical procedures for pancreatic head complications in CP, but the long-term results and QoL following these operations were disappointing, with high rates of late morbidity and mortality (73). Although two recently published articles have described better results (74, 75), it is generally accepted that these operations, involving the removal of healthy

adjacent organs, do not seem to be warranted in this benign disease (76), unless there is a strong suspicion of cancer (77). In the past 20 years, these operations have generally changed, with the introduction of Beger's DPPHR (49) and Frey's LPJ-LPHE (50) procedure. In both, the resection or excision of the pancreatic head is limited, but achieves reliable pain relief and allows definitive management of the pancreatitis-associated complications of the adjacent organs and an improved QoL (78, 79, 80). In the last 10 years, some important randomized studies have compared the different types of pancreatic head resection. Büchler demonstrated better pain relief and pancreatic function when DPPHR was compared with PPPD (81). Recently published articles based on the long-term follow-up of randomized trials have concluded that there was no difference as regards the mortality, QoL, pain, or exocrine or endocrine insufficiency between the two operations and also indicated that these operations are advantageous for the treatment of CP. The decision as to which procedure to choose should be based on the surgeon's experience (82, 83).

In approximately 30% of patients with CP, the disease is primarily located in the head of the pancreas, which is known to act as the "pacemaker" to trigger the inflammatory process; resection of this inflammatory mass must be regarded as pivotal in the surgical intervention (45, 46, 84). Basically, CP is a benign, but sometimes progressive disease, and the organ-preserving concept must therefore be accepted. The concept for our pancreatic head resection followed this directive and our preliminary clinical results confirmed it. The resection process removes only sufficient of the pancreatic head to guarantee the normal flow of both ductal systems (the bile and the pancreas) and to preserve the physiological gastro duodenal function.

Our data on 135 operated patients demonstrated that OPPHR is a safe operative procedure, as confirmed by the low morbidity (2.9%) and the absence of mortality among the patients in the postoperative period. An additional important feature is that the median duration of hospitalization was only 8.5 days. In the mean follow-up period of 4.1 years (range 0.5 to 7.0), 116 patients became complaint-free (89%), while 14 had moderate symptoms and the body weight increased significantly by a mean of 11.3 kg (range 4-28). Readmission was required for 11 of the 130 patients (8.4%) as a consequence of relaparotomy (bilio-digestive bypass) or conservatively treated pancreatitis. The late mortality was 3.7%: 5 patients died. The preoperative and postoperative endocrine function remained in almost the same stage.

Pain relief and improvement of the QoL after surgery for CP in the patients were assessed by using the EORTC QLQ-C30. The completed questionnaires before and after the surgical treatment were evaluated in 105 patients (78%). Other patients were not included in the study

because of incomplete data, or the lack of cooperation, or the data on the patients were not available. The median pain score decreased by 91% ($P<0.001$) after surgery. No patient suffered frequent pain attacks and only 10% of the patients mentioned moderate pain occasionally. During the follow-up, the median global QoL improved by 100%. Apart from the cognitive functioning, the physical status, working ability, and emotional and social functioning all improved significantly ($P<0.05$).

5.4., In spite of the good postoperative results, following the OPPHR, some of our patients claimed about recurrent symptoms. We therefore decided to investigate patients with CP in the view of cytokines to get the answer whether there is some prognostic factor or tendency in the development of this disease. Moreover the question was raised whether CP can be treated really successfully.

No correlation was found between the genotypes and the histological varieties of chronic pancreatitis. We investigated the frequency of the TT genotype in patients with chronic pancreatitis, relative to that in healthy controls, and also compared the genotypes between patients treated medically and those undergoing surgery. The latter patients were regarded as a „severe" group, with a considerable progression of the disease. The differences in TT genotype frequency proved significant between the operated group and the controls, and between the operated and the non operated group. The frequency of the TT genotype was relatively high among the medically treated patients, but only as a tendency without statistical significance.

This means that chronic pancreatitis patients who do not need surgery rather carry the „protective" C allele, while the TT genotype seems to be a risk factor for surgery. It is noteworthy, that reoperation was necessary within 3 years in 8 patients; all of them were TT homozygote. The highest TGF-β concentrations (5.2 - 7.4 ng/ml) were detected in the plasma of these patients.

The TGF-β1 plasma levels were significantly increased among the chronic pancreatic patients overall as compared with the group of healthy blood donors (3.98 ±1.26 ng/ml vs. 2.1 ± 0.85 ng/ml). Higher concentrations of TGF-β1 were detected in the plasma of the subjects with the TT and TC genotypes as compared with those with the CC genotype both among the patients and among the controls (Table2). The frequency of high producers (TT) was higher among the patients with chronic pancreatitis than among the controls (Table 3), and the TGF-β1 levels differed in the patient and control groups (Table 2). It is tempting to speculate that in

the „high producer" patients the inflammatory stimuli resulted in elevated levels of TGF-β1, which further increased the fibrotic processes in the pancreatic tissue.

The frequency of the T/C genotype was significantly higher in both groups of patients than in the controls (58 %, and 58% vs. 40%). All the successfully treated patients carried the genotypes of T/C or C/C with low serum levels of TGF-β1. (Fig.8.)

In conclusion, it is very likely that the TGF-β1 polymorphisms contribute to the genetic susceptibility to chronic pancreatitis. The TGF-β1 genetic polymorphism with higher TGF-β1 production appeared relevant among the patients with chronic pancreatitis, who underwent surgery; particularly, when reoperations were necessary. The prognostic value of TGF - β1 polymorphism and the associated TGF-β1 levels should be determined in a future follow-up study on a larger series of patients.

From this point of view, if we would like to answer whether *Can we treat patients with chronic pancreatitis successfully?* - The answer is: hopefully YES (if we are prepared to consider also the immunological risk factors…)

6. SUMMARY

1.)   The correlation between the degree of pancreatitis and the changes in the serum levels of TNF-α and IL-6 were investigated in two experimental acute pancreatitis models in rats. In both experimental models elevated TNF-α and high IL-6 levels were observed, but in PBDL group the increase in cytokine levels were about 10 fold. The results led to correlations between the serum IL-6 and TNF-α level and the biochemical and morphological severity of acute pancreatitis. These data suggest that IL-6 and TNF-α participate in the pathogenesis of acute pancreatitis

.

2.)   Low dose CCK-8 injections resulted in pancreas regeneration following pancreas resection in rats. The serum levels of IL-6 and TGF-β1 were increased significantly on days 7 and 14 following resection; and an elevated TGF-β1 expression in the pancreatic tissue was also observed. These findings indicate that IL-6 and TGFβ-1 may play a regulatory role in the regeneration of the pancreas.

3.)   A safe procedure has been developed for duodenum -preserving (organ-preserving) pancreatic head resection. The long-term follow-up clearly reveals that this resection is a safe and effective procedure for definitive control of the complications of CP.

4.)   There was a higher frequency of the TT genotype of TGF-β-1 + 869 Single Nucleotide Polymorphism, with a concomitantly higher TGF-β1 level in the plasma of patients with chronic pancreatitis than in healthy population. The number of TT homozygotes differed significantly between patients who underwent surgical intervention and the controls (OR = 4) and even between the surgical patients, and those treated medically (OR = 2.54). It seems that the TT genotype of + 869 TGF-β1 might be a risk factor for the development of a severe form of CP, and could serve as a prognostic sign for any further surgical intervention or even repeat surgery.

7. ACKNOWLEDGEMENTS

8. REFERENCES

1. Kusske AM, Rongione AJ, Reber HA. Cytokines and acute pancreatitis. *Gastroenterology.* 1996; 110:639-42.

2. Xia Q, Jiang JM, Gong X, Chen GY, Li L et al. Experimental study of Tong Xia purgative method in ameliorating lung injury in acute necrotizing pancreatitis. *World J Gastroenterol.* 2000; 6:115-8.

3. Granger J, Remick D. Acute pancreatitis: models, markers, and mediators. *Shock.* 2005 Dec;24 Suppl 1:45-51.

4. Jaster R, Emmrich J. Crucial role of fibrogenesis in pancreatic diseases. *Best Pract Res Clin Gastroenterol*. 2008;22(1):17-29.

5. Vonlaufen, A. Apte, MV Imhof BA Frossard JL.:The role of inflammatory and parenchymal cells in acute pancreatitis *J Pathol* (2007) DOI: 10.1002/path.2231

6. Dinarello CA. Proinflammatory cytokines. *Chest.* 2000; 118:503-8.

7. 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.

8. Gross V, Leser HG, Heinisch A, Schölmerich J. Inflammatory mediators and cytokines-new aspects of the pathophysiology and assessment of severity of acute pancreatitis? *Hepatogastroenterology.* 1993 Dec;40(6):522-30.

9. Uomo G, Manes G. Risk factors of chronic pancreatitis. *Dig Dis*.2007;25(3):282-4.

10. Mariani A, Testoni PA. Is acute recurrent pancreatitis a chronic disease? *World J Gastroenterol.* 2008 Feb 21;14(7):995-8.

11. Shimizu K.Pancreatic stellate cells: molecular mechanism of pancreatic fibrosis. *J Gastroenterol Hepatol*. 2008 Mar;23 Suppl 1:S119-21.

12. Witt H, Apte MV, Keim V, Wilson JS. Chronic pancreatitis: challenges and advances in pathogenesis, genetics, diagnosis, and therapy.*Gastroenterology*. 2007 Apr;132(4):1557-73.

13. Vilcek J. The cytokines: An Overview. in Thomson A (ed). *The cytokine handbook, San Diego (USA), Academic Press, Book.* 1998; 1-20.

14. Tracey KJ, Cerami A. Tumor necrosis factor: a pleiotropic cytokine and therapeutic target. *Annu Rev Med.* 1994; 45:491-503.

15. Bazzoni F, Beutler B.The tumor necrosis factor ligand and receptor families. *N Engl J Med.* 1996 Jun 27;334(26):1717-25.

16. Margetts PJ, Kolb M, Yu L, Hoff CM, Holmes CJ et al. Inflammatory cytokines, angiogenesis, and fibrosis in the rat peritoneum. *Am J Pathol*. 2002; 160(6):2285-94.

17. Vanden Berghe T, Denecker G, Brouckaert G, Vadimovisch Krysko D, D'Herde K, et al. More than one way to die: methods to determine TNF-induced apoptosis and necrosis. *Methods Mol Med.* 2004; 98:101-26.

18. Zimmermann KC, Green DR. How cells die: apoptosis pathways. *J Allergy Clin Immunol.* 2001; 108(4 Suppl):S99-103.

19. Zanotti S, Kumar A, Kumar A. Cytokine modulation in sepsis and septic shock. *Expert Opin Investig Drugs.* 2002; 11(8):1061-75.

20. Bhatia M, Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *J Pathol.* 2004; 202(2):145-56.

21. Ferrero E, Zocchi MR, Magni E, Panzeri MC, Curnis F et al. Roles of tumor necrosis factor p55 and p75 receptors in TNF-alpha-induced vascular permeability. *Am J Physiol Cell Physiol.* 2001; 281(4):C1173-9.

22. Cohen J. The immunopathogenesis of sepsis. *Nature.* 2002; 420:885-91.

23. Heinrich,P.C. Castell, J.V., Andus, T.: Interleukin-6 and acute phase response. *Biochem.J.*1990. 265: 621-636.

24. Horn,F., Henze, C., Heidrich, K.: Interleukin-6 signal transduction and lymphocyte function. *Immunobiology* 2000, 202: 151-167.

25. Hirano,T., Akira,S., Taga,T., Kishimoto,T.:Biological and clinical aspects of interleukin-6 *Immunol.Today* 1990. 11: 443-449.

26. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor ß in human disease. *New Engl J Med.* 2000; 342:1350-8.

27. Prud'homme G.J.: Pathobiology of transforming growth factor beta in cancer, fibrosis, and immunologic disease, and thrapeutic considerations. *Lab. Invest.* 2007. Aug. 1-15.

28. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-ß. *Annu Rev Immunol.* 1998; 16:137-61.

29. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ et al. Targeted disruption of the mouse transforming growth factor-ß1 gene results in mulltifocal inflammatory disease. *Nature.* 1992; 359:693-9.

30. Selman M, King TE, Pardo A. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med.* 2001; 134:136-51.

31. Bissell DM, Roulot D, George J. Transforming growth factor-β and the liver. *Hepatology.* 2001; 34:859-867.

32. Kim SJ, Jeang KT, Glick AB, Sporn MB, Roberts AB. Promoter sequences of the human transforming growth factor-beta 1 gene responsive to transforming growth factor-beta 1 autoinduction. *J Biol Chem.* 1989; 264:7041-5.

33. Wood NA, Thomson SC, Smith RM, Bidwell JL: Identification of human TGF-beta1 signal (leader) sequence polymorphisms by PCR-RFLP. *J Immunol Methods.* 2000; 234:117-22.

34. Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ et al. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantation.* 1998; 66:1014-20.

35. Arkwright PD, Laurie S, Super M, Pravica V, Schwarz MJ et al. TGF-beta(1) genotype and accelerated decline in lung function of patients with cystic fibrosis. *Thorax.* 2000; 55:459-62.

36. Hinke V, Seck T, Clanget C, Scheidt-Nave C, Ziegler R et al. Association of transforming growth factor-beta1 (TGFbeta1) T29 --> C gene polymorphism with bone mineral density (BMD), changes in BMD, and serum concentrations of TGF-beta1 in a population-based sample of postmenopausal german women. *Calcified Tissue Intern.* 2001; 69:315-20.

37. Holweg CT, Baan CC, Niesters HG, Vantrimpont PJ, Mulder PG et al. TGF-beta1 gene polymorphisms in patients with end-stage heart failure. *J Heart Lung Transplant.* 2001; 20:979-84.

38. Warle MC, Farhan A, Metselaar HJ, Hop WC, Perrey C et al. Cytokine gene polymorphisms and acute human liver graft rejection. *Liver Transpl.* 2002; 8:603-11.

39. Xaubet A, Marin-Arguedas A, Lario S, Ancochea J, Morell F et al. Transforming growth factor-beta1 gene polymorphisms are associated with disease progression in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2003; 168:431-5.

40. Li B, Khanna A, Sharma V, Singh T, Suthanthiran M et al. TGF-beta 1 DNA polymorphisms, protein levels, and blood pressure. *Hypertension.* 1999; 33:271-5.

41. Cambien F, Ricard S, Troesch A, Mallet C, Generenaz L et al. Polymorphisms of the transforming growth factor-beta 1 gene in relation to myocardial infarction and blood pressure. *Hypertension.* 1998; 28:881-7.

42. Syrris P, Carter ND, Metcalfe JC, Kemp PR, Grainger DJ et al. Transforming growth factor-ß1 gene polymorphisms and coronary artery disease. *Clin Sci.* 1998; 95:659-7.

43. Wang XL, Sim AS, Wilcken DE: A common polymorphism of the transforming growth factor-beta 1 gene and coronary artery disease. *Clin Sci.* 1998; 95:745-6.

44. Wu L, Chau J, Young RP, Pokorny V, Mills GD et al. Transforming growth factor-ß1 genotype and susceptibility to chronic obstructive pulmonary disease. *Thorax.* 2004; 59:126-9.

45. Beger HG, Schlosser W, Poch B, Gansague F. Inflammatory mass in the head of the pancreas. In: Beger HG, Warshaw AL, Büchler MW, *et al.* eds. *The Pancreas.* Oxford, London: Blackwell Science, 1998: Vol. 1., 757-60.

46. Warshaw AL. Conservation of pancreatic tissue by combined gastric, biliary, and pancreatic duct drainage for pain from chronic pancreatitis. *Am J Surg* 1985; 149: 563-69.

47. Greenlee HB, Pronz RA, Aranha GV. Long-term results of side–to-side pancreaticojejeunostomy. *World J Surg* 1990; 14:70-76.

48. Traverso LW, Longmire WP. Preservation of the pylorus during pancreatico-duodenectomy. *Surg Gynecolog Obstet* 1978; 146: 959-62.

49. Beger HG, Krauttzberger W, Bittner R, Büchler M, Limmer J. Dudenum-preserving resection of the head of the pancreas in patients with severe chronic pancreatitis. *Surgery* 1985; 97: 467-73.

50. Frey CHF, Child ChG, Frey W. Pancreatectomy for chronic pancreatitis. *Ann Surg* 1976; 184: 403-14.

51. Lampel M, Kern HF Acute interstitial pancreatitis in the rat induced by excessive doses of a pancreatic secretagogue. *Virchows Arch A Pathol Anat Histol.* 1977 Mar 11;373(2):97-117.

52. Takács T, Czakó L, Jármay K, Hegyi P, Pozsár J, Marosi E, Pap A, Lonovics J. Time-course changes in pancreatic laboratory and morphologic parameters in two different acute pancreatitis models in rats. *Acta Med Hung.* 1994;50(1-2):117-30.

53. Ceska M, Birath K, Brown B. A new and rapid method for the clinical determination of alpha-amylase activities in human serum and urine. Optimal conditions. *Clin Chim Acta.* 1969 Dec;26(3):437-44.

54. Espevik T, Nissen-Meyer J. A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. *J Immunol Methods.* 1986 Dec 4;95(1):99-105.

55. Aarden LA, De Groot ER, Schaap OL, Lansdorp PM. Production of hybridoma growth factor by human monocytes. *Eur J Immunol.* 1987 Oct;17(10):1411-6.

56. Keim V, Teich N, Moessner J: Clinical value of a new fecal elastase test for detection of chronic pancreatitis. *Clin Lab* 2003; 49 (5-6): 209-215.

57. Beger HG, Krautzberger W, Bittner R, Büchler M, Limmer J: Duodenum-preserving resection of the head of the pancreas in patinets with severe chronic pancreatitis. *Surgery* 1985; 97: 467-473.

58. Farkas Gy, Leindler L, Daróczi M, Farkas Gy, Jr: Prospective randomised comparison of organ-preserving pancreating head resection with pylorus-preserving pancreaticoduodenactomy. *Langenbecks Arch Surg* 2006; 391: 338-342.

59. Perrey C, Pravica V, Sinnott PJ, Hutchinson IV: Genotyping for polymorphisms in interferon-gamma, interleukin-10, transforming growth factor-beta 1 and tumor necrosis factor-alpha genes: a technical report. *Transplant Immunol* 1998; 6:193-197.

60. Farkas G, Leindler L, Daróczi M, Farkas Jr. G. Reply to the letter by Markus W. Büchler et al. on our publication „Prospective randomized comparison of organ-preserving pancreatic head resection with pylorus-preserving pancreatoduodenectomy". *Langenbecks Arch Surg* 2007;392:117.

61. Löser C, Möllgaard A, Fölsch UR. Fecal elastase1; a novel, highly sensitive and specific tubeless pancreatic function test. *Gut* 1996;39:580-586.

62. Farkas G, Leindler L, Daróczi M, Farkas Jr. G. Organ-preserving pancreatic head resection in chronic pancreatitis. *Br J Surg* 2003;90:29-32

63. Aaronson NK, Ahmedzai S, Bergmann B, Bullinger M, Cull A, Duez NJ, Filiberti A, Flechtner H, Fleishman SB, de Haes JC. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instruments for use in international clinical trials in oncology. *J Natl Cancer Ins* 1993;85:365-376.

64. Bloechle C, Izbicki JR, Knoefel WT, Kuechler T, Broelsch Ce. Quality of life in chronic pancreatitis: results after duodenum-preserving resection of the head of the pancreas. *Pancreas* 1995;11:77-85.

65. Saluja A, Saito I, Saluja M, Houlihan MJ, Powers RE, Meldolesi J, Steer M. In vivo rat pancreatic acinar cell function during supramaximal stimulation with caerulein. *Am J Physiol.* 1985 Dec;249(6 Pt 1):G702-10.

66. Ohshio G, Saluja A, Steer ML. Effects of short-term pancreatic duct obstruction in rats. *Gastroenterology.* 1991 Jan;100(1):196-202.

67. Lerch MM, Saluja AK, Rünzi M, Dawra R, Saluja M, Steer ML. Pancreatic duct obstruction triggers acute necrotizing pancreatitis in the opossum. *Gastroenterology.* 1993 Mar;104(3):853-61.

68. 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 Jan;34(1):41-5

69. 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 Feb;76(2):177-81.

70. Gress T, Müller-Pillasch F, Elsässer HP, Bachem M, Ferrara C, Weidenbach H, Lerch M, Adler G. Enhancement of transforming growth factor beta 1 expression in the rat pancreas during regeneration from caerulein-induced pancreatitis. *Eur J Clin Invest*. 1994 Oct;24(10):679-85.
.

71. Sohn TA, Campbell KA, Pitt HA, Sauter PK, Coleman JA, Lillemoe KD, Yeo CJ, Cameron KD. Quality of life and long-term survival after surgery for chronic pancreatitis. *J Gastrointest Surg* 2000;4:355-365.

72. Belina F, Fronek J, Ryska M. Duodenopancreatectomy versus duodenum-preserving pancreatic head excision for chronic pancreatitis. *Pancreatology* 2005;5:547:552.

73. Howard JM. Surgical treatment of chronic pancreatitis. In: Howard JM, Jordan GL Jr., Reber HA, editors, *Surgical Diseases of the Pancreas*. Philadelphia: Lea and Febiger, 1987, pp 496-521.

74. Huang JJ, Yeo CJ, Sohn TA, Lillemoe KD, Sauter PK, Coleman JA, Hruban RH, Cameron JL. Quality of life and outcomes after pancreaticoduodenectomy. *Ann Surg* 2000; 231:890-898.

75. Sakorafas GH, Farnell MB, Nagorney DM, Sarr MG, Rowland CM. Pancreatoduodenectomy for chronic pancreatitis. Long-term result in 105 patients. *Arch Surg* 2000;135:517-524.

76. Yamaguchi K, Yokohata K, Nakano K, Ohtani K, Ogawa Y, Chijiiwa K, Tanaka M. Which is a less invasive pancreatic head resection: PD, PPPD, or DPPHR? *Dig Dis Sci* 2001;46:282-288.

77. Witzigmann H, Max D, Uhlmann D, Geissler F, Schwarz R, Ludwig S, Lohmann T, Caca K, Keim V, Tannapfel A, Hauss J. Outcome after duodenum-preserving pancreatic head resection is improved compared with classic Whipple procedure in the treatment of chronic pancreatitis. *Surgery* 2003;134:53-62.

78. Beger HG, Schlosser W, Friess HM, Büchler MW. Duodenum-preserving head resection in chronic pancreatitis changes the natural course of the disease. A single-center 26-year experience. *Ann Surg* 1999;230:512-523.

79. Falkoni M, Bassi C, Casseti L, Mantovani G, Sartori N, Frulloni L, Pederzoli P. Long-term results of Frey's procedure for chronic pancreatitis: a longitudinal prospective study on 40 patients. *J Gastrointest Surg* 2006;10:504-510.

80. Aspelund G, Topazian MD, Lee JH, Andersen DK. Improved outcomes for benign disease with limited pancreatic head resection. *J Gastrointest Surg* 2005;9:400-409.

81. Büchler MW, Friess H, Muller MW, Wheatley AM, Beger HG. Randomized trial of duodenum-preserving pancreatic head resection versus pylorus-preserving Whipple in chronic pancreatitis. *Am J Surg* 1995;169:65-69.

82. Frey CF, Mayer KL. Comparison of local resection of the head of the pancreas combined with longitudinal pancreaticojejunostomy (Frey procedure) and duodenum-preserving resection of the pancreatic head (Beger procedure) *World J Surg* 2003;27:1217-1230.

83. Strate T, Taherpour Z, Bloechle C, Mann O, Bruhn JP, Schneider C, Kuechler T, Yekebas E, Izbicki JR. Long-term follow-up of a randomized trial comparing the Bager and Frey procedures for patients suffering from chronic pancreatitis. *Ann Surg* 2005;241:591-598.

84. Köninger J, Friess H, Müller M, Wirtz M, Martignioni M, Büchler MW. Duodenum-preserving pancreas head resection: An operative technique for retaining the organ in the treatment of chronic pancreatitis. *Chirurg* 2004;75:781-788.