Acute pancreatitis, from local defense to remote organ injury

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Acute Pancreatitis

From Local Defense to Remote Organ Injury

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The great tragedy of science:
The slaying of a beautiful hypothesis by an ugly fact

Thomas Henry Huxley

Basic research is like shooting an arrow into the air and,

Where it lands, painting a target.

Homer Burton Adkins

I'm tired of all this nonsense about beauty being skin deep.

That's deep enough. What do you want, an adorable pancreas?

Jean Kerr

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II. Akbarshahi H, Axelsson JB, Said K, Malmstrom A, Fischer H, Andersson R: TLR4 dependent heparan sulphate-induced pancreatic inflammatory response is IRF3-mediated. *Journal of Translational Medicine* 2011, 9:219.

III. Akbarshahi H, Menzel M, Posaric Bauden M, Rosendahl A, Andersson R: Enrichment of murine CD68+ CCR2+ and CD68+ CD206+ lung macrophages in acute pancreatitis-associated acute lung injury. *PLoS One* 2012, 7:e42654.

IV. Akbarshahi H, Sam A, Chen C, Rosendahl A, Andersson R: Early activation of TGF-Beta/Smad2 signaling in the lungs of a murine acute pancreatitis model. *Manuscript 2013*

Abbreviations

ALI	Acute lung injury
AP	Acute pancreatitis
CCL2	Chemokine (C-C motif) ligand 2
DAMP	Damage-associated molecular pattern
ECM	Extracellular matrix
HS	Heparan sulfate
HSPG	Heparan sulfate proteoglycan
IL	Interleukin
IRF3	Interferon regulatory factor 3
KC	Keratinocyte-derived chemokine
LPS	Lipopolysaccharide
MCP-1	Monocyte chemotactic protein-1
MIP-2	Macrophage inflammatory protein-2
MODS	Multi-organ dysfunction syndrome
MyD88	Myeloid differentiation primary response 88
PAMP	Pathogen-associated molecular pattern
PBS	Phosphate buffered saline
PG	Proteoglycan
PRR	Pattern recognition receptor
SAP	Severe acute pancreatitis
SIRS	Systemic inflammatory response syndrome
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-α
TRAM	TRIF-related adaptor molecule
TRIF	TIR-domain-containing adapter-inducing interferon

Popularized Summary in Swedish

Bukspottkörteln är belägen i övre delen av buken och producerar flera enzymer som är nödvändiga för matspjälkningen och dessutom hormoner som insulin som kontrollerar blodsockernivåerna. Bukspottkörteln producerar sina enzymer och de når via bukspottkörtelgången tolvfingertarmen där de blir aktiverade. Vid inflammation i bukspottkörteln (bukspottkörtelinflammation) är produktionen av såväl enzymer som insulin nersatt och dessa enzymer kan aktiveras redan inne i bukspottkörteln, vilket leder till nedbrytning av bukspottkörteln. Akut bukspottkörtelinflammation kan orsakas av gallsten (som förhindrar flödet av bukspottkörtelsaft ut i tolvfingertarmen), alkohol, och i mindre del som följd av olycksfall, mediciner, infektioner och tumörer samt genetiska störningar.
Tillståndet orsakar smärta i övre delen av buken och denna kan vara svår och åtföljas av feber, illamående och kräkning. Den svåra formen av sjukdomen kan orsaka en påverkan på hela kroppen med lågt blodtryck, chock, organsvikt och även dödlig utgång. Akut bukspottkörtelinflammation kan också orsaka blödning och vävnadsdöd i och kring själva körteln. Så kallade milda attacker av bukspottkörtelinflammation spontanläker oftast, medan i svåra fall når enzymer från bukspottkörteln och framför allt inflammatoriska substanser ut i hela kroppen.
Bukspottkörtelgångarna är täckta med en form av bindväv (s k matrix) som utgörs av ett nätverk av icke levande vävnad som stöder cellerna. Detta matrix utanför cellerna utgörs av ett flertal olika molekyler, inkluderande äggvitor och komplexa sockerarter (polysackarider). Matrixet (stödjevävnad) ger mekanisk styrka och skydd och fungerar även som ett medium för kommunikation mellan celler, t ex via tillväxtsignalering. En viktig funktion är att utgöra en bra ”fästyta” mot celler, vilket reglerar cellfunktion, viktig för exempelvis vävnadsläkning. Heparansulfat är en del av denna s k matrix utanför cellerna, intimt bunden till cellerna i sig. I samband med akut pankreatit kan matspjälkningsenzymerna klyva av heparansulfat från sin vidfästning och därmed frisätta den i löslig form.
Vi har visat att den lösliga formen av heparansulfat kan starta en inflammation i bukspottkörteln. Denna inflammation sker via ett receptor-signaleringssystem som kallas Toll-like receptor-4 (TLR4). Våra fynd öppnar möjligheterna för att ha just blockering av denna signaleringsväg som en möjlig behandling.
Svår bukspottkörtelinflammation (utgörande cirka 15 % av samtliga fall) kan leda till organsvikt, vilket är korrelerat till intensivvård och dödlig utgång i cirka 15-20 %. Vanligaste sviktande organ är lungorna och akut lungsvikt är kopplad till

hög dödlighet. Hur den inflammatoriska processen i buken når lungorna och orsakar denna akuta lungsvikt är emellertid inte klarlagt.
I dagsläget finns ingen behandling mot de underliggande orsakerna till akut lungsvikt till följd av akut bukspottkörtelinflammation. Forskning inom området har länge begränsats på grund av brist på patientmaterial och avsaknad av bra modeller för att studera sjukdomsförloppet. De biologiska mekanismer som ligger bakom sambandet mellan akut bukspottkörtelinflammation och akut lungskada är inte vetenskapligt säkerställda. För att möjliggöra ingående studie, har vi därför satt upp en experimentell modell med god sjukdomskoppling.
Makrofager är en typ av vita blodkroppar som börjar som s k monocyter. Monocyterna produceras i benmärgen och når därifrån blodcirkulationen. I samband med en infektion eller inflammation kan dessa monocyter lämna blodcirkulationen och gå ut i vävnad och organ i kroppen där de utvecklas till makrofager. Makrofager kan exempelvis ”ta upp” och avdöda bakterier, men även rensa upp annat skadligt material som virus och bakterier. Makrofager utgör alltså ett alarmsystem att något kroppsfrämmande har ankommit och hjälper andra immunceller att identifiera denna fara.
Orsaken till att vissa makrofager når lungorna är att lungorna utgör en väg in i kroppen för infektiösa substanser, som exempelvis bakterier eller virus. Membraner och substanser i näs- och munslemhinna, samt luftvägar kan filtrera bort en del organismer, och lungans makrofager försöker avdöda andra hotande organismer. I normalsituationen innehåller lungorna flera miljoner makrofager färdiga att försvara mot infektion, men när väl en sådan inträder ökas antalet makrofager hundrafalt.
Makrofager i lungorna kan endera vara belägna på ytan av lungorna, som lungmakrofager, eller röra sig ut i de små luftvägarna (alveolerna) i lungorna där de kallas alveolära makrofager. Lungans alveoler är mycket små hålrum där kroppen utbyter frisk syresatt inandningsluft mot utandningsluft med diverse substanser som ska bort från kroppen. Vid behov kan s k lungmakrofager utvecklas till just alveolära makrofager.
Förutom att identifiera och destruera skadade celler och främmande organismer så är makrofagerna också kapabla att producera signalering till immunsystemet vad det ska göra. För att uppnå detta så måste s k antigen från de identifierade främmande mikroberna och substanserna presenteras utanför cellen.
Makrofager utgör en föränderlig grupp och kan presentera cellmarkörer beroende på i vilken mikromiljö de vistas. Vi har funnit att det finns en specifik grupp av lungmakrofager som ökar vid akut lungskada i samband med akut bukspottkörtelinflammation. Denna grupp kan också utgöra en eventuell framtida målgrupp för behandling.
Makrofager utsöndrar olika s k cytokiner (inflammatoriska proteiner) under den inflammatoriska processen. En av dem är den s k transforming growth factor-beta (TGF-β). TGF-β spelar en fundamental roll vid reglering av olika biologiska

processer som tillväxt, utveckling, balans i vävnad och inte minst reglering av immunsystemet.
TGF-β har tidigare studerats, inte minst hur den verkar under senare delen av normalisering efter lungskada, men nya fynd har visat att den också kan ha en roll i samband med den akuta lungskadan. Våra resultat visar på en tidig aktivering av TGF-β-signalering i lungorna i samband med experimentell akut pankreatit.
För närvarande finns det ingen direkt bot för akut bukspottkörtelinflammation och dess komplikationer som exempelvis akut lungskada, dvs behandlingar riktade mot de underliggande mekanismerna. Därför är behandlingskoncepten idag inriktade på att stödja olika sviktande funktioner i kroppen tills den akuta inflammatoriska processen har klingat av. En ökad kunskap om underliggande mekanismer i samband med den akuta sjukdomen kan ge helt nya möjligheter för behandling och förbättrat utfall av detta svåra sjukdomstillstånd.

Acute Pancreatitis

The Pancreas

The pancreas was believed to act as a "cushion" for the stomach protecting and support blood vessels. The pancreatic ducts were firstly demonstrated by Wirsung in 1642. Shortly after, pancreatic secretions were discovered.
The digestive action of the pancreas was discovered through emulsification of fat, proteolysis, and digestion of starch. Trypsin was isolated in 1876 and shortly afterwards pancreatic amylase and lipase were identified.

The disappearance of cellular granularity after feeding was found to occur at the same time as the enzyme activity increased in pancreatic juice, and it was concluded that the cellular granules contained digestive enzyme precursors [1].

Pancreatic digestive enzymes are synthesized in a non-active form and emptied into the duodenum, where they become activated. Amylase, trypsin and lipase are the main enzymes responsible for the digestion of carbohydrates, proteins and fats, respectively.
The pancreas is also responsible for the secretion of insulin, the main hormone regulating the blood sugar levels.

Anatomy and Histology:

The pancreas is about 25cm long, located behind the stomach. The head of the pancreas is attached to the duodenum, where it empties its digestive enzymes into there. The pancreatic duct is joined by the common bile duct, going through the head of the pancreas (Fig.1).
The pancreas is a nodular gland with similarities to the salivary glands. Connective tissue surrounds the pancreas. The pancreas contains both exocrine (about 80%) and endocrine (about 2%) parts. The endocrine part consists of the insulin producing islets of Langerhans. The exocrine pancreas, with its specialized branching ductular system, contributes to the food digestion. Acidic gastric contents entering duodenum, will be neutralized by bicarbonate rich pancreatic

juice. Spherical and tubular masses of cells form the acini in the exocrine pancreas [2].

Secretory ducts join to form intralobular ducts. These ducts have low columnar epithelial cells and by anastomosing, they will form the interlobular ducts, which are lined by a columnar epithelium.
The main pancreatic duct, which has connective tissue and elastic fibers, is formed by interlobular ducts joining together [1].

Acute Pancreatitis

Acute pancreatitis (AP) is an acute, initially nonbacterial inflammation of the pancreas. Early activation of digestive enzymes in the pancreas initiates inflammation with compromise the gland itself, its nearby tissues, and remote organs. AP is a disease with extremely different clinical expressions [3].
Clinical symptoms include acute severe abdominal pain, nausea, vomiting and fever. Two major causes of AP are gallstone diseases or heavy alcohol abuse. Other causes include medications, infections, trauma, metabolic disorders, surgery, and sometimes an underlying neoplasm. In about 10 to 15%, the cause is though unknown.

AP affects around 40/100,000 of the Western general population annually and its incidence appears to be gradually increasing, although overall mortality has remained unchanged [4]. In most of the cases (80%) the disease has a mild and self-limiting course. Severe cases have a prevalence of 20%. These cases have increased risks for significant mortality and morbidity [5]. Up to 50% of the mortality rate due to severe acute pancreatitis (SAP) happens within the first week of diagnosis. This is mainly, due to systemic inflammatory response syndrome (SIRS) and single or multiple organ dysfunction. The second peak in mortality is caused by the multi-organ dysfunction syndrome (MODS) together with septic complications [6].

Pathogenesis:

Acute pancreatitis has been thought to be initiated within the acinar cells [7]. The digestive enzymes secreted mainly get activated in the duodenum in non-pathologic situations. Possible excess of activated trypsin is inhibited by various protective mechanisms [8]. Pancreatic auto-digestion occurs once defensive mechanisms prove insufficient.
The first steps causing AP are not well understood. Trypsin can extend the inflammatory process outside the pancreas by activating other pathways, such as complement, coagulation or fibrinolysis. Microcirculatory damage and increased vascular permeability will eventually result. Free radicals, pro-inflammatory cytokines (tumor necrosis factor (TNF)-α, interleukins (ILs) 1, 6 or 8), arachidonic acid metabolites (prostaglandins, platelet activator factor, leukotrines), or lipolytic and proteolytic enzymes will be liberated [9, 10].
Few AP can lead to systemic inflammatory response syndrome (SIRS). This syndrome is believed to be caused by various pancreatic enzymes and pro-inflammatory cytokines, potentially impairing respiratory, renal and cardiac function. These situations predispose the patients to infection, with higher risk for the pancreatic necrotic tissue infection, a situation where translocation of intestinal pathogens potentially plays an important role [11, 12].

Genetic factors play a role acute pancreatitis. Their role is either increasing the susceptibility to AP development or affecting the course and chronicity of the disease.

Cationic trypsinogen gene (PRSS1) and the cystic fibrosis transmembrane conductance regulator gene (CFTR), as well as polymorphisms in SPINK1 are among the known factors which increase susceptibility. Mutations in the above mentioned genes can be responsible for the premature activation of pancreatic zymogens within the pancreas and also idiopathic chronic pancreatitis and recurrent AP. The exact mechanisms by which these mutations cause AP are not fully clear. Production of a more concentrated pancreatic juice can though be a potential mechanism [13, 14].

Local Defense

The Danger Model

An immune response can be generated by both infections and sterile tissue injury. Matzinger proposed that our immune system responds to danger, rather than mediate recognition of non-self over self [15]. Tissue injury generates endogenous molecules, called damage-associated molecular patterns (DAMPs), which along with pathogen-associated molecular patterns (PAMPs) signal danger [16-18]. Cellular Toll-like receptors (TLRs) that sense these danger signals provide molecular links between tissue injury, infection, and inflammation. There are a number of endogenous molecules which can signal through TLRs. Some of them are intracellular while others are extracellular, especially components of the extracellular matrix (ECM) [19].
Activation of TLRs has been linked to many inflammatory and autoimmune diseases including sepsis, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, type I diabetes, and multiple sclerosis. TLRs and associated signaling molecules have been identified as potential targets for treatment. Aberrant TLR activation is thought to contribute to disease with strong association to inflammation such as AP [20-23].

The degree and duration of tissue damage regulate the threshold of DAMP(s) required to induce disease. The problem with quantifying different endogenous danger signals, in addition to restricted access to patient specimens and pathological data, remains main issues [24].

Extracellular Matrix

The extracellular matrix (ECM) plays different roles in the organisms beside its classical role in stabilizing cell and tissue architecture. Of particular interest in relation to inflammatory disorders, the ECM can affect immune cell functions. ECM components can interact via receptors on the inflammatory cells and initiate an inflammatory response. These components can further be shed and released during tissue injury.

Heparan sulfate (HS) glycosaminoglycans are complex polysaccharides present in ECM at the cell-tissue-organ interface, and have crucial regulatory roles in normal physiological processes. HS is usually bound covalently to different core proteins, forming heparan sulfate proteoglycans (HSPGs). HS is found on two families of membrane-bound proteoglycans, i.e. the syndecans and glypicans.

There are four different syndecan family members, i.e. syndecan-1 to -4 [25, 26]. All cells express at least one member of the syndecan family [27], with the exception of erythrocytes. Depending on the tissue, their level of expression varies. HSPGs, such as syndecan-1, are found on the epithelial cells lining the pancreatic duct.

During tissue injury and wound healing there is accelerated shedding of the ectodomain of each syndecan, a process that is highly regulated. The interaction between syndecans and various mediators can regulate cellular adhesion, migration, survival, differentiation and proliferation [28].

In pancreatitis, activated digestive enzymes such as trypsin, elastase, and phospholipase can induce shedding or release of HS, which may further influence the disease progression.

The ECM holds many essential body functions like chemotaxis, proliferation and coagulation, and is largely made up from glycoproteins, collagen, PGs, and HS.

Pancreatic acinar cells are clearly involved in the inflammatory process and act as driving forces of inflammation, but whether they actually represent a primary inflammatory starting point is not for certain. PGs can act as passive carriers of chemokines on the cell surface, but can also be cleaved off and trigger inflammation, chemokine production and recruitment of immune cells. The pancreatic duct is lined with HS-bearing proteoglycans. In presence of proteases or bile, these can be cleaved off its membrane location.

The HS chains on extracellular domains may interact with ligands like extracellular matrix glycoproteins, collagens, pro-inflammatory mediators, and enzymes.

TLR4

The Toll gene of Drosophila was firstly identified for playing an important role in the development of the dorso-ventral pattern in embryos [29]. Afterwards, it was shown that Toll-mutant flies were prone to fungal infections [30]. This finding disclosed mechanisms behind the identification of pathogen microorganisms by the immune system. Moreover, mammalian homologues of the Toll receptor were identified one after another, and designated as TLRs [31].
TLRs, representing a small number of receptors, can detect a broad range of the pathogens through pattern recognition. Therefore, they are characterized as pattern recognition receptors (PRRs). Novel findings have shown that TLRs are not only able to identify the PAMPs, but also can sense danger signals through DAMPs.
Type I transmembrane proteins recognize bacterial and viral PAMPs in the extracellular environment (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11) or endolysosomes (TLR3, TLR7, TLR8, TLR9, and TLR10).

An increasing number of endogenous stimulators are candidate ligands of TLRs [32-34]. These activate TLR signaling thereby inducing an inflammatory response. Endogenous TLR ligands are either components of cells or induced gene products, and these include extracellular matrix components [35], such as fibronectin [36], heparan sulfate [37], biglycan [38], fibrinogen [39], oligosaccharides of hyaluronan or its fragments [40, 41].
TLR4, the first discovered TLR, is expressed on the immune cells and was shown to induce the expression of genes involved in inflammatory responses [42, 43]. These findings lead to identification of several Toll receptors in mammals and their role in inflammation [44].
TLR4 recognizes several DAMPs, including fibrinogen, and various heat shock proteins (Table 1). The TLR4 recognition of many DAMPs demands several accessory molecules. As for TLR2, CD14 is necessary for binding LPS to TLR4 dimers. Another vital protein for LPS recognition is myeloid differentiation protein-2 (MD2) [45].
There is strong data indicating that TLR4 plays an important role in experimental AP in rodents. Most studies highlight the role of TLR4 in the pancreas in AP.
The general opinion is that TLR4 is very important in the development of endotoxemia in AP.

MyD88 was the first characterized main common adaptor in the TLR signaling pathway. In addition to the general MyD88-dependent pathway, a MyD88-indepentent pathway has been identified which appears to be restricted to TLR3 and TLR4 [46, 47].

Table 1. Endogenous TLR4 activators [24]

Proteins, peptides	**HMGB1** **Fibronectin EDA** **Fibrinogen** **Tenascin-C** **Surfactant protein A, D** **β-defensin-2** **HSP60,70,72,22,GP96** **S100A8 (MRP8)** **S100A9 (MRP14)** **Neutrophil elastase** **Antiphospholipid antibodies** **Lactoferin**
Fatty acids, lipoproteins	**Serum amyloid A** **Oxidized LDL** **Saturated fatty acids**
Proteoglycans, glycosaminoglycans	**Biglycan** **Heparan sulfate fragments** **Hyaluronic acid fragments**

MyD88-dependent Pathway:

MyD88 associates with the TIR domain of TLRs, and is the main adapter in the signaling pathways of IL-1 receptor and TLRs. It recruits IL-1 receptor-associated kinase (IRAK) to TLRs and starts the signaling, which finally results in the activation of JNK and NF-κB (Fig.2) [31].

MyD88-independent Pathway:

The identification of the MyD88-indepent pathway was due to the observation of hypo-responisveness of MyD88 knockout mice to LPS. At the same time, NF-κB or the mitogen-activated protein (MAP) kinase family, were activated [48]. In addition to this, it was shown that certain cytokines, such as IP-10 and GARG16, can be induced in response to LPS in MyD88 knockout cells.

The activation of MyD88-independent pathway by LPS stimulation leads to the transcription factor IRF-3, and thereby induces IFN-β. IFN-β activates Stat1, thereby initiates induction of several IFN-inducible genes [49-51].

TLR4 was initially mainly studied because of its involvement in Gram-negative bacterial infection, but has in recent years also been associated with an increasing number of diseases. Many reports suggest its involvement in atherosclerosis, liver disease, obesity, cardiac disease, and renal disease, among others.

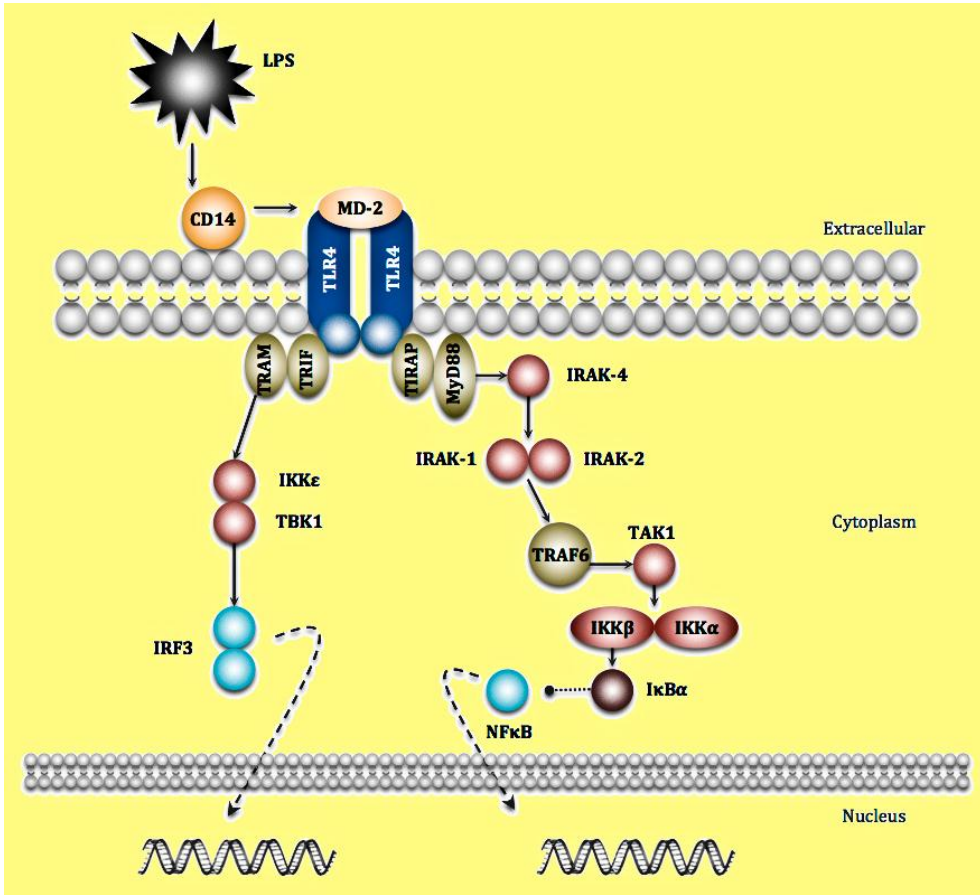

Figure 2. Schematic of TLR4 signaling cascades. Activation of TLR4 signal transduction through MyD88/TIRAP and TRAM/TRIF pathways leads to activation of innate immune system. The MyD88-dependent pathway is responsible for early-phase NF-κB and MAPK activation, which control the induction of pro-inflammatory cytokines. The MyD88-independent, TRIF-dependent pathway activates IRF3, which is required for the induction of IFN-β- and IFN-inducible genes. In addition, this pathway mediates late-phase NF-κB, as well as MAPK activation, also contributing to inflammatory responses.

Remote Organ Injury

In the majority of AP cases, the disease will follow a mild course. Severe acute pancreatitis (SAP) can be complicated with systemic release of the pro-inflammatory cytokines and pancreatic enzymes. This situation, known as SIRS, can lead to significant morbidity and mortality. The pathophysiological mechanisms behind this are, however, not fully understood [52, 53]. This syndrome can involve the lungs, kidneys and heart, among other organs, and affect their function with various degrees of dysfunction [54, 55].

Acute Lung Injury

Acute lung injury (ALI) represents the most common and earliest occurring organ dysfunction in the development of MODS in AP, where mortality is related to the number of involved organs [56]. Lung injury is accounted for 60 % of deaths within the first week of AP [55, 57].

Acute lung injury is microscopically characterized by an initial exudative phase during day 1-3 with a diffuse alveolar damage, type I pneumocyte necrosis, and influx of inflammatory cells and fluid. This is followed by a proliferative phase from about days 3 to 7 with lung repair, type II pneumocyte hyperplasia and fibroblast proliferation [58].

Underlying pathophysiological mechanisms for ALI includes a variety of derangements of the normal homeostasis, including pulmonary endothelial and epithelial barrier dysfunction. In addition, neutrophils, monocytes, and macrophages, being present both prior to “challenge” and recruited at different phases by chemokines like interleukin-8 (IL-8) and monocyte chemoattractant protein (MCP)-1, become activated (Fig.3). Pancreatitis-associated ALI is further related to specific effects on pancreatic enzymes like proteases and phospholipase A_2. Increased serum concentrations of phospholipase A_2 has been demonstrated in severe acute pancreatitis and correlates with the extent of pulmonary complications and lung injury scores [59-61].

Mechanisms involved in ALI associated with severe acute pancreatitis are complex and probably not significantly different from the ALI caused by other underlying disorders.

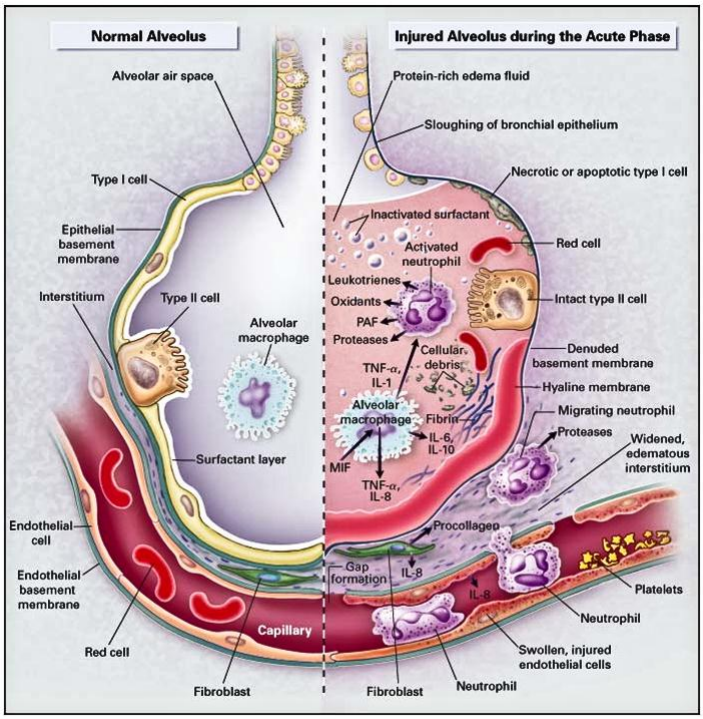

Figure 3. The normal alveolus (left-hand side) and the injured alveolus in the acute phase of acute lung injury (right-hand side). Reproduced with permission from [62], Copyright Massachusetts Medical Society.

The Role of Macrophages

Activation of different cell populations contributes to the generation of systemic inflammatory mediators. Macrophages are specialized phagocytes that reside in the lymphoid and non-lymphoid tissues, clear effete host cells and molecules, and defend against infection by their broad range of pathogen-recognition receptor and their ability to produce inflammatory cytokines [18].
In particular, peritoneal macrophages, alveolar macrophages and Kupffer cells, activated during different stages of severe acute pancreatitis, have been implicated to contribute to disease progression [63-66]. Macrophages can be activated depending on microenvironment through different activation pathways, resulting in marked phenotypic heterogeneity [67, 68].

In humans and mice, monocytes are divided into two major subsets that either specifically traffic into inflamed tissues or, in the steady state, constitutively maintain tissue macrophage populations. Through their development into resident macrophages, monocytes contribute both to host defenses and to tissue remodeling and repair [69].
Macrophages are a heterogeneous population based on their anatomical location and specialization of function. Furthermore, in addition to the macrophage heterogeneity based on their residency in various organs, macrophage heterogeneity is also observed in a single organ. The macrophage populations in the tissues are renewed through: 1) the entry of new monocytes and 2) through local proliferation to maintain a steady state [70].

Macrophages in the Lungs:

Alveolar macrophages have the capacity to secrete a vast number of chemokines, cytokines, growth factors and reactive oxygen and nitrogen species. Hence, they possess multiple pro- and anti-inflammatory roles in the respiratory tract. The activation of the alveolar macrophages leads to the recruitment of leukocytes from the circulation, including monocytes, neutrophils and T lymphocytes.
Alveolar and interstitial macrophages play distinct roles in the acute lung injury associated with acute pancreatitis. Alveolar macrophages promote an early inflammatory response, whereas interstitial macrophages appear to have a protective role to resolve the inflammation [71]. Increased nitric oxide synthesis related to induction of iNOS in alveolar macrophages has been suggested to contribute to the acute lung injury secondary to pancreatitis [72, 73]. The use of phospholipase A2 (PLA2) inhibitors indicate that this enzyme could be involved in the activation of alveolar macrophages and generation of nitric oxide [74]. PLA2 has further been shown to regulate cytokine production by monocytes/macrophages as well as phagocytosis and superoxide (O_2^-) generation by neutrophils [75].

Inhibition of NF-κB activation may reverse the lung injury in acute necrotizing pancreatitis by inhibiting the release of inflammatory mediators by alveolar macrophages [76]. Neutrophil recruitment to the lungs during AP is in part mediated by chemotactic mediators (TNF-α and MIP-2) released by activated alveolar macrophages [72]. In AP, endothelial cells, neutrophils and macrophages release platelet activating factor (PAF), which has been implicated as a key mediator in the progression of AP, leading to complications and high mortality rates.

Alveolar macrophages play a role in the removal of particles and microorganisms from the alveolar space, while interstitial macrophages limit inflammation, fibrosis, and antigen presentation [77]. Some of the sequestered monocytes migrate into the interstitium or the alveolar spaces and differentiate into mature macrophages [78].

Macrophages play a major role in tissue repair, including the clearance of apoptotic debris as a result of primary injury and the inflammatory response. The tissue repair process the production of transforming growth factor (TGF)-β by macrophages is accelerated following phagocytosis of apoptotic debris, suggesting potential anti-inflammatory and pro-fibrotic effects of this phenomenon [79].

TGF-β

Normal homeostasis between the cells and the ECM is essential. Various cytokines regulate this interaction. The TGFβ is a member of a family of growth factors that regulate cell proliferation, differentiation, embryonic development, wound healing, immune responses, apoptosis and angiogenesis. Every cell in the body produces this cytokine and has its receptors. Three TGF-β isoforms exist (TGF-β1, TGF-β2, and TGF-β3) that are expressed tissue specifically.
TGF-β1 and 3 are expressed in the early phases of morphogenesis, while TGF-β2 has a later expression in mature epithelium. The three isoforms have different binding affinities for the receptors.
The TGF-β isoforms contain a pro-peptide region, the latency associated peptide when synthesized. Most of TGF-β will be stored in ECM in complex with a protein called latent TGF-β binding protein as the third member of this complex. TGF-β can be released from the inactive complex by the multifunctional matrix glycoprotein thrombospondin-1 and plasmin-mediated cleavage of the complex. The process of activation is an important step [80].
TGF-β regulates multiple cellular processes by binding to three high-affinity cell-surface receptors known as types I, II, and III. TGF-β type I receptor gets phosphorylated by the type II receptor and this originates the signal. Ligand binding induces the formation of a heteromeric complex of type I and II receptors. Given the dimeric nature of the ligands, each monomer might contact one type I receptor and one type II receptor, thereby generating a heterotetrameric receptor complex [81].

The proteins of the SMAD family are the first identified substrates of type I receptor kinases. The transduction of the signals from the receptor to target genes in the nucleus happens through SMADs [81, 82]. The founding member of the SMAD family is the product of the *Drosophila* gene *Mad* (*mothers against dpp*) [83].

There are three distinct types of Smads based on their functional and structural characteristics:
a) Receptor regulated (R-) Smads
b) Common mediator (Co-) Smads
c) Inhibitory Smads (I-) Smads

R-Smads, i.e., Smad1, Smad2, Smad3, Smad5, and Smad8, are the direct substrates for the type I receptor [84]. Activated R-Smads subsequently makes a complex with Smad4 (Co-Smad) and translocates to the nucleus where it participates in transcriptional regulation of target genes (Fig.4) [85, 86].
The I-Smads, i.e. Smad6 and Smad7 can antagonize the stimulatory effects of the activation of R-Smads by direct competition, proteosomal degradation or

dephosphorylating of the receptors [87-91]. TGF-β stimulation rapidly induces the expression of I-Smads [92, 93].

Aberrant regulation of TGF-β ligand availability or subsequent activation of downstream TGF-β signaling pathways can be responsible for various disorders. For example, TGF-β overexpression is often observed in cancer, fibrosis and inflammation. The effects can be due to the imbalance in the interaction of cells and ECM [80].
Gene mutations at different levels of the signaling pathway are also responsible for human disorders. These mutations can be found in cancer, hereditary chondroplasia and hereditary hemorrhagic telangiectasia [83].

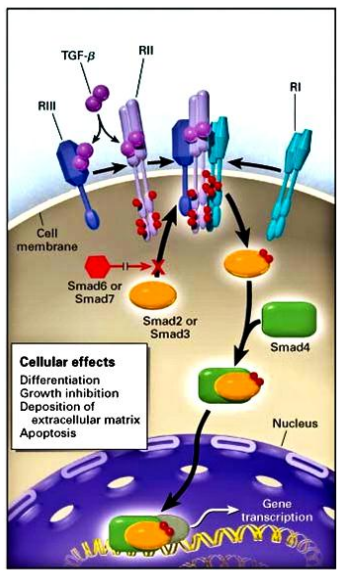

Figure 4. Mechanism of signal transduction mediated by TGF-β.Reproduced with permission from [80], Copyright Massachusetts Medical Society.

Results

The aim of this thesis was to study acute pancreatitis from local defense to remote organ injury.
Specifically, the focus has been on two main tasks:

- In what manner ECM components and the immune system are cooperating for pancreatic tissue well-being.
- To shed light on some aspects of the role the innate immunity plays in developing remote organ injury in AP.

Local Defense

Acute pancreatitis facilitates a pathological situation with different types of active enzymes in the pancreatic tissue which can lead to the cleaving off and shedding of various components of extracellular matrix including HS.
We investigated the inflammatory response to HS in the pancreatic tissue and further looked at signaling pathway involved in this process.
In the first study (paper I), rats were randomized in three different groups which were subjected to intraductal (pancreatic duct) infusion of HS, LPS or PBS. We reported that intraductal infusion of HS and LPS, both induce host defense reactions in the pancreas by involving different patterns of cell-recruitment. In the pancreas of HS-infused rats we showed an early infiltration of ED-1-positive monocyte/macrophage cells. Neutrophils were recruited to the pancreatic tissue at later time points. This recruitment pattern was different from that observed in the pancreas of LPS-infused rats, where neutrophils preceded monocyte/macrophage recruitment. This was further confirmed by chemokine expression.
These findings imply that the etiology of pancreatic inflammation may influence how the subsequent events will develop.
In order to define whether the HS-induced inflammatory response was TLR4-mediated and determine the downstream signaling adapters involved, the second study was carried out (paper II). To benefit from genetically modified animals this study was done in mice. Specific knockout mice for TLR4 and key TLR4 signaling adapters (MyD88 and IRF-3), as well as wild-types were utilized in the study. Similar to the first study the effects of intraductal (pancreatic duct) infusion of HS were compared to LPS and PBS at different time points.

The intraductal HS infusion induced neutrophil infiltration and myeloperoxidase (MPO) activation after 9 hours in the pancreas of the wild-type mice. LPS-infused mice had an earlier and more pronounced neutrophil recruitment and MPO activity compared to HS-infused ones.

The major chemoattractants for neutrophils (KC or CXCL1) and monocytes/macrophages (MCP-1 or CCL2) were increased in the LPS-infused animals whereas there was no rise in the CXCL1 levels in the HS-infused group.

We demonstrated that the HS-induced inflammatory response is TLR4-mediated. The MPO activity in the pancreas of $TLR4^{-/-}$ mice was significantly less than what was seen in the wild-type group. This was further confirmed by using a synthetic lipid A analogue (eritoran) to block TLR4. The MPO activity of HS-infused wild-type mice was abolished by pretreatment with eritoran.

We showed that the inflammatory response to HS involves both myeloid differentiation 88 (MyD88)-dependent and independent pathways. Interestingly, HS-induced inflammation was interferon regulatory factor 3 (IRF3) dependent, indicating a role of interferon pathway in host inflammation by HS (Fig.5).

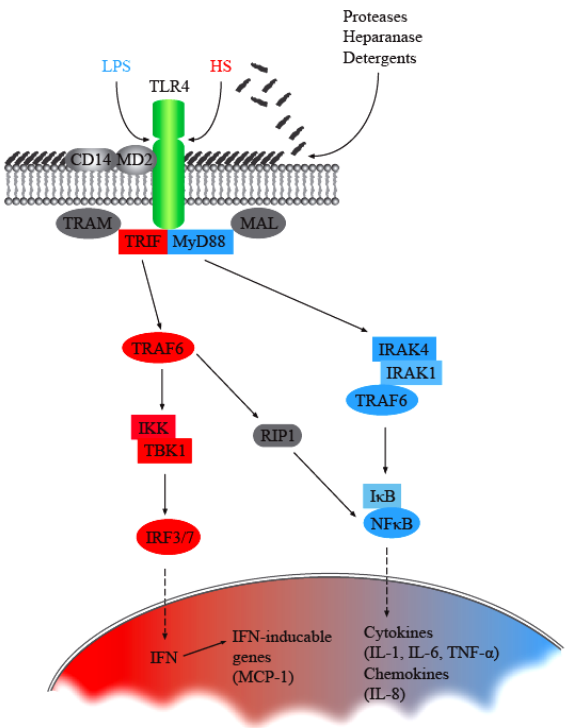

Figure 5. Signaling events following triggering of TLR4 by HS and LPS.

Remote Organ Injury

Animal Model:

It is important for experimental studies to use animal models with sufficient similarity to the corresponding human condition. The murine pancreatic ductal ligation model is a newly described experimental model mimicking severe gallstone-induced AP, characterized by systemic inflammation, multi-organ dysfunction and substantial mortality. In addition, ligation-induced AP in mice is associated with significant pulmonary inflammation and dysfunction.
Compared to existing models of ALI in AP, this model more closely resembles human gallstone impaction causing AP [94]. Some of the existing experimental models (e.g. cerulein-induced acute edematous pancreatitis) are not usually associated with ALI, systemic inflammation, multi-organ dysfunction or mortality [95]. Existing models associated with systemic inflammation, multi-organ dysfunction and mortality (e.g. CDE-diet-induced AP, retrograde duct injection-induced AP) differ in that they do not closely mimic human etiologies of the disease [10, 95, 96].

ALI and Macrophages:

Murine biliary and pancreatic ductal ligation resulted in acute pancreatitis (paper III). This was accompanied by systemic inflammatory response and inflammation in the lungs. Recruitment of neutrophils along their important chemoattractant (CXCL1) was seen in both organs.
Macrophages are key cells determining the severity of acute lung injury. Distinct populations of macrophages in the lungs undergo dramatic changes in both number and phenotype during the development and resolution of lung injury.
Our results showed an interesting pattern of expression of macrophage cell markers. We studied different phenotypes of macrophages with regard to the expression of two main cell markers (F4/80 and CD68) in our model. Our data showed an enriched population of $CD68^{+}$ $F4/80^{-}$ in the lungs of the animals with acute pancreatitis.
CCL2 is the important chemoattractant for macrophages and acts through its cell surface receptor (CCR2). The CCL2/CCR2 axis has a central role in the recruitment and activation of macrophages. The lungs of the animals with acute pancreatitis had an enriched $CD68^{+}$ $F4/80^{-}$ $CCR2^{+}$ population.

Macrophages can be further classified into either classically activated macrophage with an M1-like or alternatively activated macrophages with an M2-like phenotype. The M1-like macrophages are generally $CD11c^{+}$ cells and associated with a pro-inflammatory Th1 immune response. This pro-inflammatory phenotype produces reactive oxygen intermediates and is involved in bacterial defense. In

contrast, the M2-like macrophages are associated with a more anti-inflammatory Th2 immune response, involved in collagen production, tissue repair and fibrosis. Up-regulation of CD206 (macrophage mannose receptor) distinguishes the alternative activation from classic activation. The enriched CD68+ F4/80- population in the lungs of the animals with acute pancreatitis had increased CD206+ macrophages.

ALI and TGF-β Signaling:

Activated pulmonary macrophages secrete TGF-β. TGF-β has mainly been studied during the late phases of tissue repair. However, indications of an early involvement of TGF-β in the initial phases of ALI exist. Several TGF-β–inducible genes have been found to be dramatically increased as early as 2 days after the induction of injury [97]. TGF-β through different mechanisms can contribute to the lung injury.

We have shown that TGF-β1 levels increased in the lungs of animals with acute pancreatitis as early as at 9 hours (paper IV). The increase in the level of TGF-β1 was associated with an enhanced expression of TGFβR1 (ALK-5).
Downstream of the TGF-β signaling pathway the inhibitory Smad7 was increased early after the acute pancreatitis. This increased level was not seen at the later time point. The reduced level of Smad7 was accompanied by an increased nuclear translocation of phosphorylated Smad2 indicating an activated TGF-β signaling transduction.

Discussion

DAMPs and Immune System

In 1994 Polly Matzinger wrote [15]:

*"*For many years immunologists have been well served by the viewpoint that the immune system's primary goal is to discriminate between self and non-self. I believe that it is time to change viewpoints and, in this essay, I discuss the possibility that the immune system does not care about self and non-self, that its primary driving force is the need to detect and protect against danger, and that it does not do the job alone, but receives positive and negative communications from an extended network of other bodily tissues."

A surveillance system for monitoring the status of the animals' cells and the invasion of microorganisms is essential for survival. An optimal system requires danger signals, cells which can respond to the signals and various signaling pathways. At the final stages these signals should be conveyed as physiological responses.

DAMPs are not only generated by sterile tissue injury, but also by various pathogens causing tissue damage. They are released upon injury and by activation of TLRs, they induce an inflammatory response aimed for tissue repair. This response is beneficial, as long as there is an appropriate amount of released DAMPs. Excessive amount of damage signals can pass the "beneficial" levels and start a pathological state. This happens in various diseases where excessive inflammation plays a key role in pathogenesis, including rheumatoid arthritis, cancer, and atherosclerosis.

In addition to the excessive amounts of DAMPs, TLR activation can also initiate a positive feedback loop by starting the inflammatory response. Inflammation and recruitment of inflammatory cells may lead to more tissue destruction and consecutively more DAMPs release. This means the low levels of DAMP can start a beneficial response which is resolvable and physiological. On the other hand, higher levels of DAMPs can initiate a damage chain reaction (Fig.6) [24].

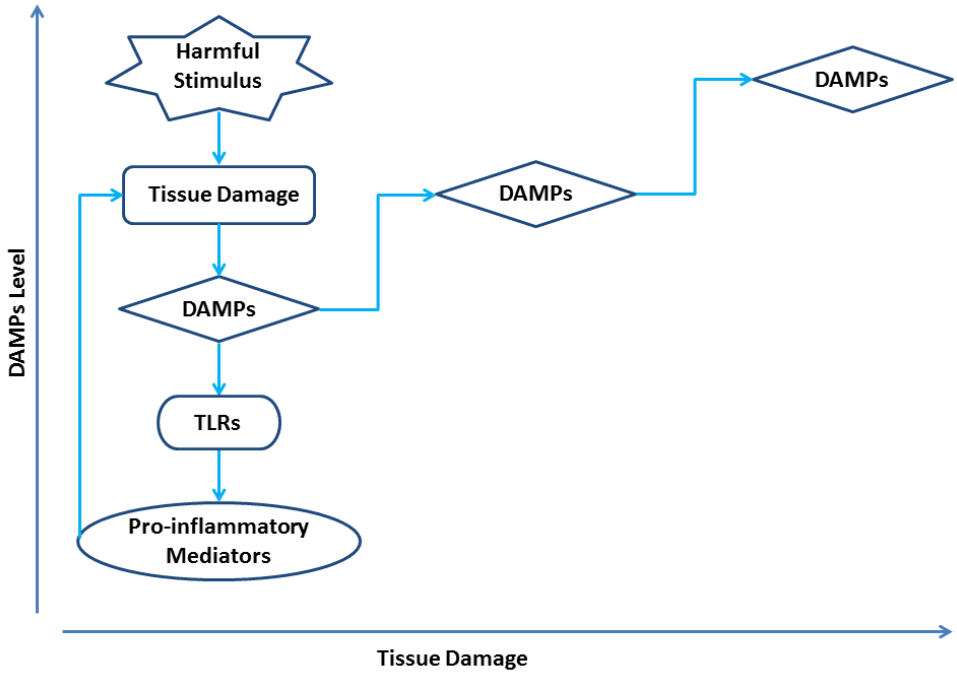

Figure 6. The "damage chain reaction".

A good example of this is the ischemic injury. Cellular injury due to ischemia initiates release of DAMPs and restoration of the blood supply brings in the inflammatory cells which will contribute to more tissue injury and DAMPs release. TLRs contribute to the development of atherosclerosis and Alzheimer's disease through sensing of damage signals in the form of oxidized lipoproteins [98]. Hence, TLR2 and TLR4 antagonists may be useful in these conditions to prevent an overactive immune response [99].

Pancreas is an ideal environment for DAMPening inflammation. The exocrine pancreas is responsible for secreting various enzymes into the duodenum to digest food. Several lines of defense, such as non-optimal PH and proteinase inhibitors, prevent from the premature activation of the zymogens.

Acute pancreatitis is an inflammatory disease of the pancreas, which leads to the pathological activation of digestive enzymes within the pancreas, instead of in the small intestine. Activated enzymes start the process of auto-digestion of the pancreatic tissue. Several pancreatic enzymes are able to act on the extracellular matrix components. Specifically heparinases and proteases are able to cleave off heparan sulfate from its anchors and form soluble HS.

HS starts an inflammatory response in the pancreatic tissue, which includes enhanced levels of chemoattractants and recruitment of inflammatory cells.
This inflammatory response is through TLR4 signaling. Both MyD88-dependent and independent pathways are involved in this signaling.

The response to HS is a weaker response compared to the response towards the well-known TLR-4 ligand, LPS. As discussed earlier, this can be beneficial for tissue repair to induce a resolvable, physiological immune response.
It is clear in the pathological states which the tissue injury is more severe and the amount of DAMPs passes the threshold of a beneficial and resolvable inflammatory response, DAMPs can contribute to the pathological destruction of the pancreas with a mechanism similar to the DAMP chain reaction.

Multi-organ Dysfunction Syndrome

Acute pancreatitis varies in its clinical presentation ranging from mild self-limiting disease to severe pancreatitis, with potential development of both local and systemic complications [100-103]. Early death relates to organ dysfunction, and late mortality is usually due to the combination of sepsis and organ failure.
In patients with acute pancreatitis, progression to a severe course appears to be associated with the inappropriate activation of an inflammatory cascade leading to the development of a systemic inflammatory response [9, 104, 105], which in turn results in the development of multi-organ dysfunction and death. The term systemic inflammatory response syndrome (SIRS) was developed to imply the presence of the clinical response to a non-specific inflammatory insult [106]. SIRS has a precise clinical definition, which has been validated in large patient populations [107, 108].
It is widely accepted that local pancreatic inflammation is the initiating stimulus for a systemic inflammatory response. This in turn results in the development of multi-organ dysfunction and contributes to death from acute pancreatitis.

In vivo Models:

Experimental models of acute pancreatitis and remote organ injury provide the opportunity of investigating the role of inflammatory mediators and pancreatic enzymes in the pathogenesis of acute pancreatitis and remote organ injury. By using these models, the critical role of many inflammatory mediators including TNF-α, IL-1β, IL-6, PAF, IL-10, CD40L, C5a, ICAM-1, chemokines, substance P and hydrogen sulfide in acute pancreatitis and remote organ injury has been identified [109].
The ductal ligation model, which was used by us as a systemic inflammatory response model of acute pancreatitis, resembles the human disease course and is easily reproducible. The profound inflammatory response in the lungs makes this model relevant for studying the acute lung injury in acute pancreatitis.

Lung Macrophages:

Lung macrophages are divided into three groups: alveolar, interstitial and recruited macrophages. Alveolar macrophages have the important task to recognize the pathogens and start the inflammatory response as the first line of defense. Pulmonary macrophages not only have a role in the initiation of the inflammatory response, but also contribute to the resolution of inflammation and tissue repair. The resolution process has a multistep course which involves reversion of all the inflammatory steps[110].

There is a cross-talk between the macrophages and their microenvironment. In the lungs the airway epithelium has an impact on this interaction. Depending on the intensity of the pro- or anti-inflammatory mechanisms the macrophage phenotype can form. This phenomenon is called the pulmonary "*innate immune rheostat*" [111, 112]. Lung surfaces are exposed to commensals as well as pathogens. This requires a higher threshold of response from the macrophages to not impair the pulmonary gas exchange. This is achieved by regulating the microenvironment by the airway epithelial cells [110].
Our data showed an enriched population of $CD68^{+}$ $F4/80^{-}$ in the lungs of the animals with acute pancreatitis. Whether this population has a role in the pathogenesis of the disease or will play a role in the resolution of the inflammatory process in the lungs needs more investigation.
The enriched population can be due to recruitment and/or the local proliferation of the macrophages. In our study these macrophages had a higher expression of the receptor for CCL2 (CCR2), which is more in favor of recruitment.

Macrophage Polarization:

Macrophages respond to the environment signals with their plasticity and can polarize to classically activated (M1), or alternative activated (M2) phenotype.
The balance between these two phenotypes determines the fate of inflammatory response. M1 macrophages have microbicidal and phagocytic activity, while M2 macrophages can promote fibrotic and angiogenic functions [113-115]. M1 macrophages have anti-fibrotic properties by releasing CXCL10 [116], or matrix metalloproteinases [117].

Our data showed an early increase in $CD68^{+}$ $F4/80^{-}$ $CD206^{+}$ cells in the lungs of animals with acute pancreatitis. Enrichment of $CD206^{+}$ macrophages can result from either recruitment of these cells from the circulation or effect of the lung microenvironment on the residing macrophages. CCL2 is one of the cytokines that can polarize the macrophages towards an M2-like type. In our model, we have higher tissue levels of CCL2 in the lungs of the animals with acute pancreatitis.
M2-like macrophages are involved in various tissue remodeling processes, where they secrete pro-fibrotic cytokines such as TGF-β.

TGF-β in ALI:

TGF-β has three isoforms, from which TGF-β1 is secreted mostly after tissue injury [118]. The role of TGF-β in the fibrotic processes after injury to the lungs has been widely studied [119, 120]. The lungs of the patients with ARDS have a higher level of TGF-β1 or its inducible genes (pro-collagen, type III) [121, 122]. These findings raise a question about the role of TGF-β signaling in ALI. TGF-β not only participates in the late phase of acute lung injury, but also might be active early in acute lung injury and potentially could contribute to the development of pulmonary edema. Integrin-mediated local activation of TGF-β plays a role in the development of pulmonary edema in ARDS, and blocking TGF-β or its activation could be an effective treatment for this disorder. In line with these recent findings, our data showed an early activation of TGF-β signaling in the lungs of the mice with acute pancreatitis.

There was an increase in the level of TGF-β1 in the lungs of the acute pancreatitis group early on but this was in parallel with an increase in the level of inhibitory Smad7. The level of Smad7 was back to the control level after 24 hours when there was an increase in the nuclear translocation of phosphorylated Smad2. This time point was the same time point which we had the peak of the inflammatory response associated with the inflammatory cells recruitment to the lungs as well as pathological changes indicating the lung injury, in the third paper.

Future Perspectives

DAMPs and Immune System

The cross-line between beneficial inflammatory response and getting into a vicious cycle which can result in chronic inflammation is a key question in dealing with DAMP and the immune system. To some extent this depends on the level of initial tissue damage and the quantity of the released DAMPs. The other issue to be addressed is whether this threshold is specific to different DAMPs and the responsible TLR or not.
We observed a lower level of response to HS (DAMP) compared to LPS (PAMP); this is expected as being a tissue well-being monitoring system. It is unknown if in general the immune reaction towards DAMPs is milder than what we see for PAMPs, and if so what are the mechanisms with which the immune cells can recognize the DAMPs from PAMPs.
The aim of the involvement of the immune system after tissue injury is the repair process. Which brings us to the question: what are the specific properties of the DAMPs that make the TLR response to them different from PAMPs?
The structural diversity of the DAMPs makes it difficult to know if TLRs are responding to all or some.
Regarding HS and acute pancreatitis, we have to answer the issue of the specificity of the TLR response to various HS depending on their level of sulfates and the length of chain.
It is also needed to investigate if HS response through TLR4 needs any co-receptor like what is seen for hyaluronan, TLR2 and CD44.
It is also not clear whether HS binds to TLR4 or not, and if so does it bind directly or through binding to a co-receptor.

Multi-organ Dysfunction Syndrome

The link between AP and ALI is still not fully comprehended. The release of pro-inflammatory cytokines in the blood is one of suggested mechanisms as well as the release of pancreatic enzymes. Various pro-inflammatory cytokines and pancreatic enzymes have been used as a target in animal studies of AP and remote

organ injury. The findings have not been decisive in showing a vivid image of the disease.
The exocrine pancreas is a deprived organ in terms of resident innate immune cells. The level of the cytokines released in the early phases of the AP in our model is less than what we see in blood or lung tissue. The amplification of response is an important issue in the course of the disease. Inflammatory cells in the blood can be responsible for part of the cytokine "storm". The other candidates are the Kupffer cells in the liver, which can contribute to the pro-inflammatory cytokine release.
It is not clear if the link between AP and ALI is through the blood stream or/and lymphatic system. The only treatment which has been shown to have beneficial effects in humans was thoracic duct drainage, though this study has been done in a small sample population [123].
Diverting the blood flow from the pancreas towards the inferior vena cava instead of liver, via a porto-caval shunt, has decreased inflammation in the lungs [124].

Macrophages

The first issue to be addressed about the role of macrophages in AP induced ALI, is the role of extra-pulmonary populations. Two major ones are the peritoneal and hepatic (Kupffer cells) macrophages. There are some animal studies which have shown the contribution of these macrophages to SIRS and remote organ damage.
Kupffer cells are the first population, which get exposed to the blood coming from the inflamed pancreas. They can be a potential amplifier for the inflammatory response in acute pancreatitis.
Peritoneal macrophages are in direct contact with the pancreas and their secretions get rapidly absorbed to the bloodstream.
To what extent these two populations are responsible for ALI in AP is not fully comprehended.
When it comes to the role pulmonary macrophages in ALI, there are several issues which should be considered. Despite all the important findings in the field of pulmonary macrophages there are still crucial issues to be addressed. Macrophages, as plastic immune cells are forming the first line of defense in both alveoli and pulmonary tissue, and are responsible as the first cells to react to pathogens and potential harmful factors. This makes the regulatory mechanisms of their microenvironment of great value. The interaction between the airway epithelium, macrophages and other immune cells is of utmost interest.
Macrophages can initiate, promote and stop the inflammatory responses. The regulation of switching between these different phenotypes and adopting a new

one is complex. Whether the phenotype of the macrophages is merely decided by their microenvironment, or is lineage-confined is another issue to be addressed.
An increase in number of the macrophages can be reached by local proliferation and recruitment from the bloodstream. The role of each of these new to the tissue macrophages should be investigated.
Regarding the findings in the third paper, there are some questions yet to be answered in future, including:
The role of the enriched $CD68^{+}$ $F4/80^{-}$ population; Whether this enriched population is playing a role in the pathogenesis of the ALI in AP or is responsible for the initiation of tissue repair has not been answered in our study.
Our findings were mostly in favor of the recruitment of these cells to the lungs. If this is the case, we should investigate the mechanisms behind the recruitment of the specific phenotype of the macrophages to the lung tissue.

TGF-β

It was previously mentioned that TGF-β is not only playing a role in the fibrotic process after lung injury, but also can contribute to ALI by its effect on the endothelial and epithelial cells. TGF-β can also initiate the tissue repair.
The main issue here is: whether there are regulating mechanisms behind keeping the balance between the detrimental and beneficial effects?
It is not clear whether this is due to imbalance in secretions and/or activation of different isoforms of TGF-β or the involved signaling pathway or transcription of specific target genes in the nucleus.
We also have to address the alternative signaling pathways beside the one we have studied, to have a more clear image.
Investigating the target genes and their products which are modified in our model will help us to understand better the role of TGF-β in ALI due to AP.

Clinical Aspects

DAMPs and TLRs

Previously, we discussed the damage chain reaction and its role in inflammatory diseases. This opens the doors for usage of blocking the TLR response to DAMPs as a therapeutical approach.
This has been shown in different inflammatory diseases with the result of amelioration of the disease [125-128].
The sensitive issue is the role of TLRs in the immune defense against the PAMPs. By generally blocking the TLR response, we endanger the body in response to pathogens. This is a delicate task, i.e., choose the unique targets which are responding to tissue injury and not pathogens. Blocking the specific co-receptors of DAMPs might be the alternative way.
We showed that the downstream response to DAMPs can be different from the response to PAMPs. This gives an opportunity to use those targets or final products of the signaling pathway for therapeutical purposes.
Understanding of the specificity of immune response to DAMPs can help us to combat the inflammatory diseases with much less side effects.
The other approach is the prevention of release and accumulation of DAMPs in the tissue. This can be achieved by targeting the enzymes responsible for shedding of different ECM components [129-132].

ALI

Cytokines and Chemokines:

In the pathogenesis of respiratory complications following AP, cytokines and chemokines, in particular IL-1β, IL-6, IL-8, IL-18 and TNF-α, play major roles [133]. *In vivo* studies have used various cytokine and chemokines as potential therapeutic targets but none of them has proven beneficial in clinical studies. We still lack effective treatment directed at the underlying pathophysiological mechanisms and treatment in general is merely organ supportive [134].

Macrophages as a Potential Therapeutic Target in ALI:

Pulmonary macrophages play a role in various acute and chronic lung diseases [135], and the number of lung macrophages increase upon toxic exposure [136-138].

Several *in vivo* studies have focused on targeting pulmonary macrophages for therapeutical purposes. Inhibition of macrophages [139, 140], macrophage ablation [141-144], or PAF antagonists to block the activation of alveolar macrophages[145], had some beneficial effects in reducing the lung injury. Pulmonary macrophages are rapidly activated to release cytotoxic and pro-inflammatory mediators upon tissue injury [146, 147]. Anti-inflammatory steroids can improve lung damage induced by e.g. ozone or silica, bleomycin [148-150].

Protection against lung injury has also been described in leukopenic rats as well as rats depleted of alveolar macrophages [137, 151-153]. CCR4-deficient mice, knockout mice lacking the gene for CD40 and IL-18 knockout mice, which do not generate M1-like macrophages, had an abrogated lung injury in response to bleomycin [154-156]. Activating macrophages enhances acute lung injury induced by endotoxin or radiation, as well as bleomycin-induced tissue damage [157, 158].

On the other hand, defects in M2-like macrophage recruitment which is observed in mice lacking CCR2, make them hypersensitive to hyperoxia-induced acute lung injury [159].

Macrophage polarization has also been used by *ex vivo* polarization towards M2-like cells and subsequently transfer these cells into animals with acute pancreatitis [160]. This approach results in a reduction of histological score and in levels of circulating amylase. However, the long time required to obtain polarized macrophages remain and may limit its approach as a therapeutical strategy.

Together, these studies reveal that the development of lung injury is accompanied by a dramatic change in macrophages. The above referred studies demonstrate a role for macrophages in determining the severity of the acute lung injury, opening a novel area of investigation to identify new therapies to treat patients with acute lung injury [134].

Targeting TGF-β in ALI:

TGF-β and its signaling pathways have been used to develop different therapeutical interventions for various kinds of diseases including fibrosis and cancer [161].
Furthermore experimental studies have shown beneficial effects of targeting TGF-β in ALI. Blocking of TGF-β with monoclonal antibody caused decreased mRNA levels of pulmonary pro-inflammatory cytokines in a hemorrhage-induced model of ALI. These mice had a better histological score as well. These results suggest that TGF-β has an important role in hemorrhage-induced acute lung injury [162].
Integrin αvβ6 is an integrin which activates latent TGF-β [163]. Mice lacking αvβ6 were protected against pulmonary edema though they had an enhanced inflammatory response compared to wild types in a bleomycin-induced ALI model [164].
Administration of a TGF-β antagonist prevented the development of pulmonary edema in both bleomycin and endotoxin-induced model of ALI. [165].
All the above studies point out that targeting the TGF-β signaling pathway can represent a potential strategy to be used in the future treatment of ALI.

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