

Interleukin-1 Family: Key Regulators of Immune Response and Fibrosis in Acute and Chronic Pancreatitis

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List of abbreviations

ADM	Acinar-to-Ductal Metaplasia
AP	Acute Pancreatitis
ASC	Apoptosis-associated Speck-like Protein Containing a CARD
ATP	Adenosine Triphosphate
BMDMs	Bone Marrow-Derived Macrophages
CAF	Cancer-Associated Fibroblasts
CAPS	Cryopyrin-Associated Periodic Syndromes
CARS	Compensatory Anti-Inflammatory Response Syndrome
CCK	Cholecystokinin
CCK-8	Cell Counting Kit-8
CCR2	C-C Chemokine Receptor Type 2
CEL	Carboxyl Ester Lipase
CEL	Carboxyl Ester Lipase
Col1a1	Collagen Type I Alpha 1 Chain
Col3a1	Collagen Type III Alpha 1 Chain
CP	Chronic Pancreatitis
CPA1	Carboxypeptidase A1
CTRC	Chymotrypsin C
DAMPs	Damage-Associated Molecular Patterns
DIRA	Deficiency of Interleukin-1 Receptor Antagonist
Dnases	Deoxyribonucleases
ECM	Extracellular Matrix
ER	Endoplasmic Reticulum
ER	Endoplasmic Reticulum
FDA	U.S. Food and Drug Administration
FGF2	Fibroblast Growth Factor 2
FMF	Familial Mediterranean Fever
HSC(s)	Hepatic Stellate Cell(s)
ICAM-1	Intercellular Adhesion Molecule 1
IL-1	Interleukin 1
IL-13	Interleukin-13
IL-13R	Interleukin-13 Receptor
IL-18	Interleukin-18
IL-1R/ IL-1R1	Interleukin-1 Receptor / Interleukin-1 Receptor Type 1
IL-1R3	Interleukin-1 Receptor Accessory Protein (also called IL-1RAcP)
IL-1Ra	Interleukin-1 Receptor antagonist
IL-1 α	Interleukin 1 alpha

IL-1 β	Interleukin 1 beta
IL-33	Interleukin-33
IL-36	Interleukin-36
IL-37	Interleukin-37
IL-38	Interleukin-38
IL-4	Interleukin-4
IL-4/IL-4R α	Interleukin 4 / Interleukin 4 Receptor Alpha
IL-4R	Interleukin-4 Receptor
IL-6	Interleukin 6
LPS	Lipopolysaccharide
M1	Classically Activated Macrophages
M2	Alternatively Activated Macrophages
MyD88	Myeloid Differentiation Primary Response 88
NETs	Neutrophil Extracellular Traps
NF κ B	Nuclear Factor kappa B
NLRP3	NOD-, LRR- and pyrin domain-containing protein 3
PAMPs	Pathogen-Associated Molecular Patterns
PDGF	Platelet-Derived Growth Factor
PP cells	Pancreatic Polypeptide cells
PRRs	Pattern Recognition Receptors
PRSS	Protease, Serine 1 (Cationic Trypsinogen)
PSC(s)	Pancreatic Stellate Cell(s)
RA	Rheumatoid Arthritis
Rnases	Ribonucleases
ROS	Reactive Oxygen Species
SPINK1	Serine Peptidase Inhibitor Kazal Type 1
TGF β	Transforming Growth Factor Beta
TGF β R	Transforming Growth Factor Beta Receptor
Th2	T-helper 2
TLR	Toll-Like Receptor
TNF- α	Tumor Necrosis Factor Alpha
TNF α	Tumor Necrosis Factor alpha
VCAM-1	Vascular Cell Adhesion Molecule 1
α SMA	Alpha Smooth Muscle Actin

List of Units

g	gram
%	percent
°C	degrees Celsius
d	day
h	hour
mg	milligram
mg/ml	milligrams per milliliter
min	minute
mL	milliliter
mM	millimolar
ng	nanogram
ng/ml	nanograms per milliliter
nm	nanometer
OD	optical density
pg	picogram
pH	potential of hydrogen (acidity/alkalinity)
rpm	revolutions per minute
sec	second
U/ μ L	units per microliter
v	volume
μ g	microgram
μ L	microliter
μ m	micrometer
μ M	micromolar
μ m	micrometer

1. Introduction

1.1 The Pancreas

The pancreas is a vital organ with both exocrine and endocrine functions, playing a central role in digestion and metabolism. Anatomically, it is divided into the head, body, and tail. It is located retroperitoneally in the upper abdomen, posterior to the stomach, and extends toward the spleen (1,2).

The exocrine component of the pancreas, comprising approximately 85% of the tissue, is specialized in producing and secreting digestive enzymes and bicarbonate. Acinar cells are the primary units responsible for the synthesis and secretion of different classes of digestive enzymes. Lipases and amylases are secreted in their active forms while proteases are synthesized as inactive precursors or zymogens and stored in specialized vesicles known as zymogen granules and are subsequently secreted into the pancreatic duct for delivery to the duodenum. In addition to these enzymes, DNases and RNases are also produced by the acinar cells and released into the duodenum. The synthesis of proteases as inactive precursors, such as trypsinogen and chymotrypsinogen is a protective mechanism of the pancreas which prevents premature proteolytic activity within the acinar cells, protecting the organ from self-digestion. Upon reaching the duodenum, trypsinogen becomes activated to trypsin by the proteolytic processing of the N-terminal activation sequence by enteropeptidase (enterokinase), a membrane-bound enzyme located on the brush border of the duodenal epithelium. Active trypsin catalyzes the activation of other digestive enzymes, including chymotrypsin and elastase thereby initiating a downstream protease cascade that ensures efficient nutrient breakdown (1–3).

The endocrine portion of the pancreas is organized into clusters known as the islets of Langerhans, which constitute about 1-2% of the pancreatic mass. These islets contain several cell types, including beta cells that secrete insulin, alpha cells that produce glucagon, delta cells that release somatostatin, and PP cells that produce pancreatic polypeptide. This intricate cellular arrangement enables the pancreas to maintain glucose homeostasis by regulating blood sugar levels through the coordinated secretion of hormones (1–3).

1.2 Pancreatitis

Pancreatitis is the most common cause of gastrointestinal disease-related hospitalizations, resulting in substantial morbidity, mortality, and socioeconomic impact (4–8). It is most frequently caused by gallstones, which can obstruct the biliary or pancreatic ducts, or by excessive alcohol consumption (9). In addition to gallstones and alcohol use, hypertriglyceridemia is also associated with an increased risk of developing acute pancreatitis (10), along with epigenetic factors such as smoking and obesity. Protective factors include a balanced diet, which may slightly reduce the risk of developing pancreatitis. This inflammatory condition of the pancreas can present as either acute or chronic, with varying durations and severities (1).

1.2.1 Acute pancreatitis

Acute pancreatitis (AP) is a severe abdominal inflammatory condition characterized by parenchymal edema, necrosis, and, in some cases, the formation of pseudocysts, abscesses, hemorrhages, and inflammatory cell infiltration (1). The disease spectrum of AP ranges from mild, self-limiting episodes to severe cases with potentially life-threatening complications (1). Mild acute pancreatitis (AP) has a very low mortality rate, typically less than 1%. However, for severe acute pancreatitis, the death rate ranges from 10% to 30%, depending on whether necrosis is sterile or infected. In the United States, approximately 210,000 patients are hospitalized annually due to acute pancreatitis (11). Although the majority of patients experience a mild form of the disease, approximately 20% develop severe pancreatitis, which is associated with pancreatic necrosis and systemic inflammation. Severe acute pancreatitis can result in multi-organ failure or the infection of pancreatic necrosis by commensal bacteria from the gut (6,7,12). The infection of pancreatic necrosis is a life-threatening risk factor during disease manifestation and can finally lead to severe sepsis (13). Despite progress in understanding the disease, no causative treatment for acute pancreatitis has been established to date (7). In mild forms of pancreatitis, fluid replacement, analgesics and enteral nutritional therapy are usually used. In severe cases, patients are additionally monitored in intensive care (14).

The pathogenesis of acute pancreatitis (AP) is initiated by the premature activation of digestive enzymes within pancreatic acinar cells, leading to cellular injury and subsequent

autodigestion of the pancreas by its own proteases (5,6). The initial step in this process is the intracellular activation of trypsinogen to trypsin by the lysosomal hydrolase cathepsin B. Subsequently trypsin can activate other pro-forms of digestive proteases such as chymotrypsinogen or pro-elastase and finally induce the whole protease cascade.

Beside the activation of digestive enzymes, the transcription factor Nuclear factor kappa B (NF κ B) induces a pro-inflammatory reaction of the acinar cell by the production of cytokines and chemokines and recruits the first immune cells to the site of damage. Necrotic acinar cells release damage-associated molecular patterns (DAMPs), such as free DNA, histones, and adenosine triphosphate (ATP). These DAMPs are recognized by immune cell receptors, eliciting a robust proinflammatory response (6). DAMPs act on infiltrating immune cells via Toll-Like-receptor signaling and enhance the pro-inflammation further more (6,8). This inflammatory response includes the secretion of proinflammatory chemokines and cytokines (IL-1 α , IL-1 β , IL-6, and TNF α), which recruit additional leukocytes to the site of inflammation and activate them. The inflammatory response has also an impact on the local tissue damage and determines the severity of the pancreatitis (Fig.1). Leukocytes, particularly macrophages and neutrophils, are prominently recruited to the site of injury (5,6). Neutrophils contribute to pancreatic damage by the release of reactive oxygen species (ROS) (15) and the formation of neutrophil extracellular traps (NETs) (16).

In contrast to neutrophils, monocytes infiltrate the damaged pancreas in significantly higher numbers (6,7,8). The primary role of macrophages is defensive, characterized by phagocytosing necrotic tissue and clearing cellular debris (5,6). However, macrophages also contribute to pancreatic damage through the release of TNF α , which can directly induce acinar cell necroptosis, the major form of cell death during AP (17,18). Macrophages react on local circumstances and can act in different directions. During the acute phase of pancreatitis, macrophages become activated by DAMPs and pro-inflammatory cytokines and differentiate into classically activated macrophages (M1), which are characterized by the release of pro-inflammatory cytokines and enhance the local and systemic immune response.

In the later stages of the disease, when necrosis is removed and the release of DAMPs is diminished, macrophages differentiate into a regenerative phenotype (alternative activated

macrophages, M2), which contribute to wound healing and regeneration by the release of growth factors.

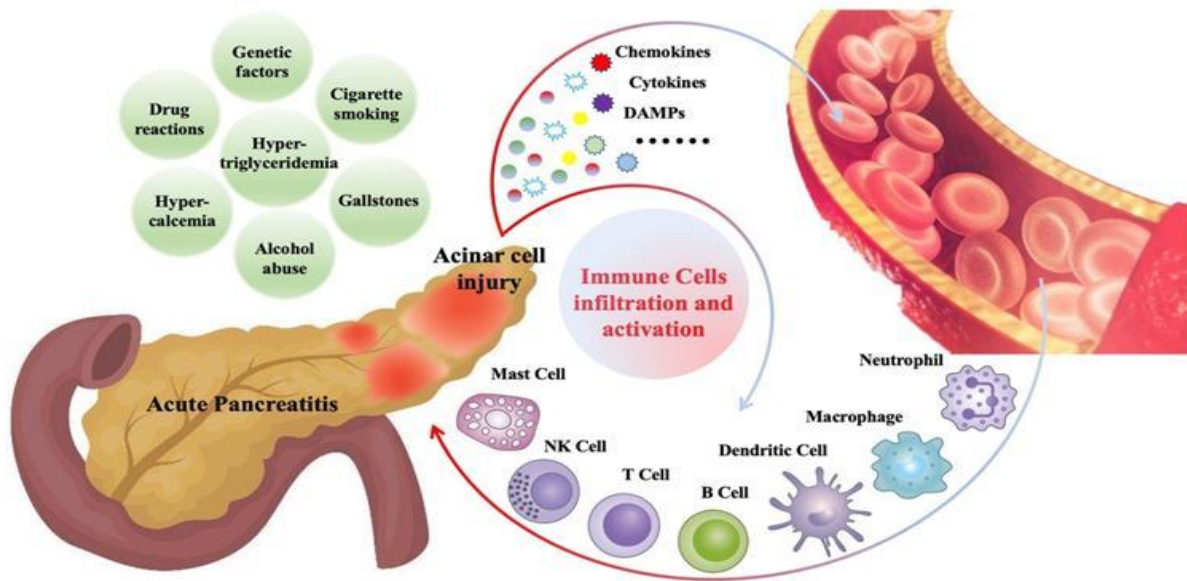


Figure 1: Immune response during acute pancreatitis (adapted from Peng et al, 2021)(19).

1.2.2 Chronic pancreatitis

Chronic pancreatitis is a multifactorial, fibroinflammatory disorder characterized by recurrent episodes of acute pancreatitis (AP) and pancreatic inflammation. Approximately 20–30% of patients with AP experience a recurrence, and around 10% progress to chronic pancreatitis (CP) (20,21). The likelihood of progression from AP to CP is higher in individuals with ongoing alcohol consumption or smoking, as well as those with genetic predispositions, such as hereditary pancreatitis (22). In addition to alcohol abuse, various genetic mutations are associated with an increased risk for chronic pancreatitis. Most of these mutations are found in genes encoding pancreatic proteases, such as cationic trypsinogen (PRSS1), the trypsin inhibitor SPINK1, or chymotrypsin C (CTRC). Mutations like R122H (23) and A16V in the human PRSS1 gene are believed to enhance the activity of trypsin or prevent its degradation by other proteases, such as chymotrypsin C (24). These mutations contribute to pancreatitis by increasing trypsin activity. Mutations in SPINK1 are also thought to affect trypsin activity by diminishing the inhibition of active trypsin (25). In addition to mutations in the trypsin activation pathway, several other mutations have been identified that lead to enhanced ER stress and damage to acinar cells. ER stress is mainly induced by misfolded mutations in highly

expressed genes, such as CEL (carboxyl ester lipase) (26) and CPA1 (carboxylpeptidase A1) (27).

This chronic form of the disease, marked by recurrent episodes of acute pancreatitis (AP), leads to extensive fibrotic tissue replacement, resulting in chronic pain and progressive, irreversible damage to the pancreas. Chronic pancreatitis is characterized by acinar cell atrophy, ductal distortion and/or obstruction, and, ultimately, exocrine and endocrine pancreatic insufficiency. As a consequence, patients may develop diabetes mellitus, experience a reduced quality of life, and have a shorter life expectancy. Furthermore, patients with chronic pancreatitis have a higher risk of developing pancreatic cancer, which has a very poor prognosis and one of the highest mortality rates among solid tumors (20–22). The management of chronic pancreatitis is challenging, primarily focusing on the treatment of complications. Symptomatic therapies aim to alleviate pain, manage malabsorption, and treat diabetes mellitus; however, most patients continue to experience symptoms despite limited supportive interventions. The incidence and prevalence of chronic pancreatitis are increasing, and currently, no curative treatment exists to halt its progression or reverse the condition (8,21,28,29).

During acute episodes of pancreatitis, necrotic areas in the pancreas are replaced by fibrotic tissue. This process is referred to as the necrosis-fibrosis sequence (30). As the disease progresses, the amount of fibrosis continually increases, preventing the regeneration of exocrine tissue, which ultimately results in the loss of both exocrine and endocrine function. An organ-preserving therapy must address this balance between fibrosis and regeneration to prevent excessive fibrosis and allow for acinar cell regeneration.

1.3 Pancreatic stellate cells/fibroblasts

Pancreatic stellate cells (PSCs) are resident cells of the pancreas, comprising 4–7% of all parenchymal cells in the gland. PSCs share strong similarities with hepatic stellate cells (HSCs) and play a critical role in pancreatic fibrosis, much like HSCs do in hepatic fibrosis. In addition to producing extracellular matrix (ECM) components such as type I collagen, PSCs contribute to local immune responses, inflammation, angiogenesis, and both exocrine and endocrine pancreatic functions. *In vitro* studies have shown that PSC activation is triggered by growth

factors such as TGF β and PDGF (31). Other contributing factors include ethanol and its metabolites, oxidative stress, mechanical pressure, endotoxins, hyperglycemia, hypoxia, angiogenic factors, various cytokines such as IL-6, and proteases, all of which contribute to pancreatic injury and PSC activation (32–34).

In a healthy pancreas, PSCs remain quiescent and are characterized by the presence of vitamin A-containing lipid droplets in their cytoplasm (35). However, in response to pancreatic injury or inflammation, they undergo morphological and functional changes, transforming into myofibroblast-like cells—a process known as PSC activation (31,36,37). Key markers of PSC activation include increased α -smooth muscle actin (α SMA) expression, loss of vitamin A-containing lipid droplets, enhanced proliferation, migration, and ECM protein production, particularly type I collagen and fibronectin (32,33).

PSCs are well established as key mediators of pancreatic fibrogenesis. Their proliferation occurs early during pancreatic necroinflammation. In recent years, PSCs have also been recognized for their role in pancreatic repair and regeneration following acute pancreatitis, where they provide an extracellular matrix (ECM) scaffold to support acinar cell restitution (32,33).

Recent studies underscore the central role of pancreatic stellate cells (PSCs) in chronic pancreatitis (CP)-associated fibrogenesis by regulating the synthesis and degradation of extracellular matrix (ECM) proteins. The disease progresses from a pro-inflammatory state in the acute phase to tissue repair and fibrosis during the regeneration phase. PSC activation is induced by various factors, including toxic agents associated with pancreatitis (e.g., ethanol) and cytokines released from injured acinar cells or pancreas-infiltrating leukocytes such as macrophages and neutrophils (34,37,38). Among these, transforming growth factor-beta (TGF- β) secreted by alternatively activated macrophages is crucial for activating PSCs (29,39), leading to the production of extracellular matrix proteins, including type I and type III collagens, in an effort to promote wound healing and fibrogenesis. While PSC activation is essential for tissue repair, excessive and sustained activation promotes fibrosis, disrupting normal pancreatic architecture and potentially leading to the loss of both exocrine and endocrine functions (28,29).

1.4 Macrophages

The term “*Macrophage*” originates from ancient Greek and means “giant eating (phagocytosing) cell.” The name reflects one of their most important functions: macrophages phagocytose dead cells, cellular debris, germs, and pathogens. As such, they play a crucial role in inflammatory and tissue-regenerative responses triggered by various forms of tissue damage, including infections, autoimmune disorders, mechanical injuries, and toxic insults. In the case of acute pancreatitis (AP), tissue injury activates monocytes derived from the bone marrow or tissue-resident macrophages. During acute infections or tissue damage, these cells adopt a pro-inflammatory role before transitioning into a wound-healing phenotype (39). This highlights the remarkable plasticity of macrophages, which enables them to adapt to their environment and perform both pro-inflammatory and anti-inflammatory functions, as well as support wound healing (40).

Traditionally, macrophages are classified into M1 (pro-inflammatory) and M2 (anti-inflammatory and reparative) subtypes based on specific stimuli and functions. However, under physiological and pathological conditions, macrophages exhibit significant heterogeneity in both phenotype and function across different tissues (41,42). Their polarization within a given tissue is dynamically influenced by the complexity of the local microenvironment (39).

The role and phenotype of macrophages have been extensively studied in both acute and chronic pancreatitis. Pancreatitis triggers an immediate immune response to local cell injury, significantly influencing disease progression (8). Acinar cell damage, often caused by premature activation of intracellular proteases, leads to the release of damage-associated molecular patterns (DAMPs), which activate innate immune cells. This activation induces the production of pro-inflammatory cytokines and chemokines, initiating a localized immune response (8). Tissue-resident macrophages are among the first immune cells to detect DAMPs, amplifying the initial immune reaction (8). The release of cytokines and chemokines further recruits monocytes and neutrophils from the bone marrow to the damaged pancreas (8,43). The recruited monocytes differentiate into M1 macrophages in response to DAMPs via Toll-like receptor (TLR)-dependent signaling pathways (44–46). As the local immune response progresses, additional immune cells infiltrate the pancreas and contribute to inflammation by

releasing pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF- α) (6,8,47–50), interleukin-6 (IL-6) (51), interleukin-1 β (IL-1 β) (52,53) and reactive oxygen species (ROS) (8,15). On the one hand, macrophages intensify inflammation and exacerbate pancreatic damage during the acute phase; on the other hand, they are also essential for clearing necrotic tissue, thereby helping to limit further injury (54).

Later in the disease course, a shift from a pro-inflammatory to an anti-inflammatory phenotype is essential for wound healing and fibrosis regulation (8,29,39). Continuous phagocytosis of necrotic areas also reduces the release of DAMPs and prevents further polarization of macrophages toward the M1 phenotype. As the inflammatory phase transitions to recovery, macrophages shift from an M1 to an M2-like phenotype, becoming predominant during the acinar-to-ductal metaplasia (ADM) stage. In chronic pancreatitis (CP), macrophages interact with pancreatic stellate cells (PSCs), promoting M2 polarization via the IL-4/IL-4R α axis (29,39). While this shift supports tissue regeneration, persistent extracellular matrix (ECM) production by macrophages in CP can drive fibrogenesis and chronic tissue remodeling (39). Alternatively activated macrophages also display dual roles: on the one hand, they contribute to tissue regeneration (55), on the other hand, they promote progressive fibrosis of the organ (29), which ultimately leads to the loss of pancreatic function.

The immune response, and especially macrophages, determines whether acinar cell damage is repaired, resolved, or replaced by fibrotic tissue. Severe disease outcomes often result from dysregulated or imbalanced immune responses, which are associated with high morbidity and mortality (8). While the underlying triggers of pancreatitis may vary, the immune response to cell injury follows a common pattern, making it a promising target for therapeutic intervention. However, due to the complex and dynamic nature of immune regulation throughout the disease, any clinical intervention must be carefully designed to maintain an optimal balance between inflammation and resolution (8).

1.4 Interleukin-1 family

The IL-1 family comprises 11 cytokines with distinct or overlapping roles in inflammation and cancer (Fig. 2). The interleukin-1 (IL-1) family plays a central role in innate immunity, primarily by driving inflammatory responses. Cytokines of the IL1 family, especially the inflammasome-

dependent cytokines IL-1 β and IL-18, are a hallmark of M1-like macrophages (42,56). Inflammation serves as a crucial defense mechanism against pathogens but can become harmful when dysregulated. IL-1 family cytokines initiate innate immune inflammation by signaling through IL-1 family receptors (Fig.2). While their pro-inflammatory functions are predominantly associated with innate immunity, IL-1 family members also contribute to acquired immunity (57).

A key feature of these cytokines is that they are activated by proteolytic cleavage, although some are active in their precursor form, cleavage increases their activity. IL-1 α and IL-1 β , the first members identified, signal through the IL-1R receptor (58). While significant progress has been made in understanding IL-1 β 's biogenesis and its involvement in human diseases, the regulation of IL-1 α production and its bioavailability remain less explored. IL-1Ra inhibits IL-1R1, thereby blocking the actions of both IL-1 α and IL-1 β . IL-33, a dual-function cytokine released as an alarmin during cell damage, is inactivated by caspase 1. It is processed extracellularly by enzymes such as neutrophil elastase and cathepsins, which enhances its activity (59,60). IL-18, synthesized as an inactive precursor, is activated by caspase 1 and plays a role in promoting inflammation and immune responses, and can interact with the adaptive immune system (61). IL-37 is distinct within the IL-1 family, as it suppresses both innate and acquired immunity. Both precursor and processed forms of IL-37 are biologically active. IL-36 is pro-inflammatory and requires proteases like caspase 1 and furin for activation, with both precursor and cleaved forms being active. IL-38, a more recently discovered member, has anti-inflammatory properties, with its precursor form being biologically active, while caspase processing enhances its effects (57,62).

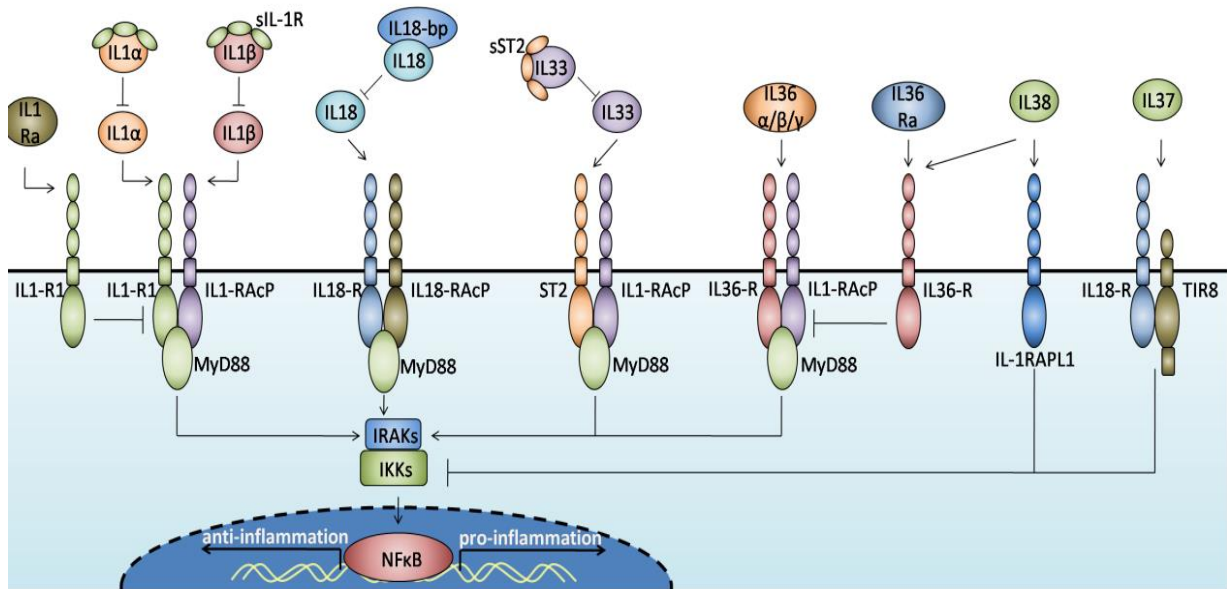


Figure 2: Interleukin-1 (IL-1) cytokines family, their corresponding receptors, and associated signaling pathways.

1.4.1 Interleukin-1α

Interleukin-1 alpha (IL-1α) is a member of the IL-1 cytokine family and is constitutively present in various cells under homeostatic conditions. It is found in keratinocytes, epithelial cells of mucosal membranes, and organs such as the liver, lungs, and kidneys. Additionally, platelets contain IL-1α (57,63,64).

IL-1α functions as a dual-purpose cytokine, playing a key role in sterile inflammation. As a dual-function cytokine, IL-1α can localize to the nucleus, where it binds DNA and regulates gene expression, while also acting extracellularly by binding to its membrane receptor to initiate signal transduction. It is retained in resting non-hematopoietic cells, including those in the gastrointestinal tract, liver, kidney, and skin. IL-1α is classified as an alarmin, meaning it is ready to function immediately upon release from damaged cells, without the need for de novo synthesis. As such, it can sense and report cellular damage in the environment when released from necrotic cells, serving as an early-warning signal. (57,63–66).

Upon tissue injury, IL-1α acts as a critical alarm signal, initiating the inflammatory response. During necrosis, damaged cells release IL-1α into the extracellular space, where it binds to the IL-1R1 receptor, triggering a cascade of signaling events. This leads to the production of chemokines and cytokines, which in turn recruit neutrophils to the site of injury. As the inflammatory response progresses, monocytes are subsequently recruited. In contrast,

apoptotic cells retain IL-1 α within the nucleus through chromatin binding, preventing its release and thereby suppressing the activation of inflammation (57,62,64).

Upon binding to IL-1R1, IL-1 α triggers the recruitment of IL-1R3 (fig.3), forming a signal transduction complex that activates MyD88 and IL-1 receptor-associated kinases, ultimately leading to NF- κ B translocation to the nucleus and activation which enhances pro-inflammatory gene expression. Independently, intracellular IL-1 α can regulate gene transcription without IL-1R1 binding (57,64,66).

IL-1 α primarily exists in its precursor form, which is biologically active but can be further processed by proteases such as calpain, granzyme B, mast cell chymase, and neutrophil elastase. While calpain cleavage is rare, it enhances IL-1 α 's inflammatory potency by increasing receptor affinity and promoting its release from cells. Additionally, caspase-1 facilitates IL-1 α secretion, further amplifying the inflammatory response (57,62,64). Pre-IL-1 α has been shown to upregulate the transcription of IL-6 and procollagen in systemic sclerosis fibroblasts, contributing to the disease's pathogenesis (64,67,68).

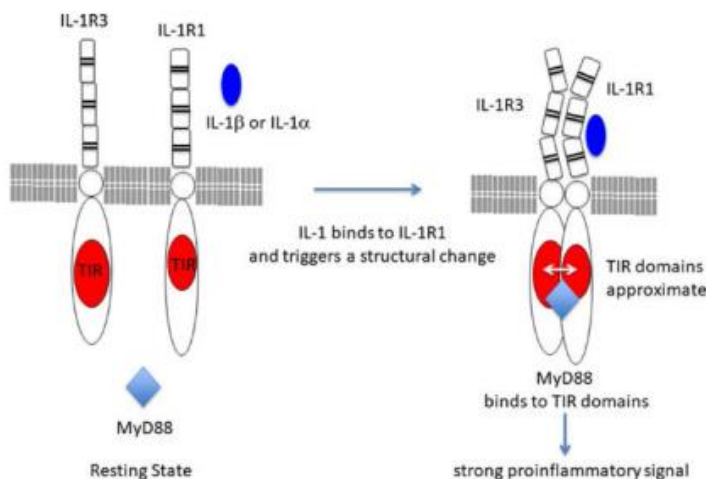


Figure 3: IL-1 α binding to its receptor and activation of the pro-inflammatory signaling pathway (adapted from Dinarello, 2018)(57).

1.4.2 Interleukin-1 β

Interleukin-1 β (IL-1 β) is a potent pro-inflammatory cytokine essential for host defense against infections and injuries. It binds to the interleukin-1 receptor type 1 (IL-1R1), the same receptor

as IL-1 α . While IL-1 β is produced by various cell types, most studies focus on its synthesis in innate immune cells, particularly monocytes and macrophages (69).

IL-1 β is initially synthesized as an inactive precursor, pro-IL-1 β , in response to pathogen-associated molecular patterns (PAMPs). These molecular motifs, recognized by pattern recognition receptors (PRRs) on macrophages, activate signaling pathways that regulate gene expression. This induction phase, known as priming, is insufficient for secretion. A secondary stimulus, either another PAMP or a damage-associated molecular pattern (DAMP) such as ATP, is required to trigger pro-IL-1 β processing and secretion (69).

Unlike IL-1 α , pro-IL-1 β is inactive, does not bind IL-1R1, and requires cleavage for activation. This cleavage is primarily carried out by intracellular caspase-1, although extracellular proteases, such as neutrophilic elastase and matrix metalloproteinase-9, can also process IL-1 β . In monocytes and macrophages, caspase-1 inhibitors block IL-1 β maturation, leading to cytosolic accumulation of its precursor (57,66). Caspase 1 should be activated to convert cytosolic pro-IL-1 β into its active form. Caspase-1 activation occurs within a multiprotein complex called the inflammasome, composed of cytosolic PRRs, adaptor proteins, and pro-caspase-1. The best-characterized inflammasome is the NLRP3 inflammasome, formed by the PRR NLRP3, the adaptor protein ASC and caspase 1 (69). The inflammasome complex maturation needs two signals for its activation. First a priming signal (LPS or DAMPs) induces the transcription of inflammasome components such as ASC, NLRP3, caspase 1 as well as gasdermin D and pro IL-1 β . A second signal is needed to induce the inflammasome complex formation and the activation of the caspase 1 which finally activates gasdermin D pore formation and pro-IL-1 β and pro-IL-18. Upon activation, caspase-1 cleaves pro-IL-1 β , allowing its maturation and subsequent release via multiple pathways, including exocytosis of secretory lysosomes, shedding of plasma membrane microvesicles, and transport through exosomes and multivesicular bodies. Additionally, pyroptosis, a caspase-1-dependent form of cell death, facilitates IL-1 β release (57,66).

In contrast to IL-1 α , the role of IL-1 β is well characterized and investigated in case of acute pancreatitis. The release of IL-1 β is initiated by DAMPs, a genetic deletion of TLR9 or the ATP receptor P2X7 results in reduced inflammasome activation accompanied by a reduced release of IL-1 β (53). The diminished release of IL-1 β results in weaker pro-inflammation and

ameliorates the disease severity. The deletion of gasdermin D also results in a blockade of IL-1 β release and thus results in a milder form of AP (70). Furthermore, serum levels of IL-18 showed a positive correlation with the disease severity in patients (71,72). Further evidence for the importance of IL-1b for the course of pancreatitis comes from an artificial, but nevertheless informative mouse model. Animals that overexpress the mature form of IL-1 β in pancreatic acinar cells develop a chronic form of pancreatitis, which ultimately leads to complete loss of function of the organ (73).

1.5 Anakinra

Anakinra, a recombinant form of the naturally occurring IL-1 receptor antagonist (IL-1Ra), was the first selective IL-1Ra approved in 2001 by the US Food and Drug Administration (FDA) (63). It remains a leading IL-1 therapeutic due to its excellent safety profile, short half-life, and multiple administration routes (63). Anakinra effectively blocks IL-1 receptor type 1 (IL-1R1), thereby inhibiting the actions of IL-1 α and IL-1 β (57).

IL-1 plays a crucial role in the pathogenesis of autoinflammatory disorders. Blocking IL-1, particularly IL-1 β , has become the standard therapy for various autoinflammatory diseases, such as deficiency of IL-1 receptor antagonist (DIRA) and Familial Mediterranean Fever (FMF), which are characterized by recurrent fevers and systemic inflammation. In these conditions, dysfunctional monocyte-macrophages drive inflammation (57).

Anakinra is approved for the treatment of rheumatoid arthritis (RA) and cryopyrin-associated periodic syndrome (CAPS) (57). In RA, it helps reduce symptoms and slow joint destruction (66). Research also explores IL-1 blockade in noninfectious chronic inflammatory conditions, including heart failure. Studies have shown that anakinra may benefit patients with heart failure, poor exercise tolerance, and systemic inflammation. Additionally, case reports have demonstrated its efficacy in treating refractory pericarditis (57).

Anakinra and other IL-1 inhibitors generally have a favorable safety profile, with no associated organ toxicities or gastrointestinal disturbances. While routine infections have been reported, opportunistic infections in anakinra-treated patients remain rare (57).

1.6 Aim of the study

Although pancreatitis can have various triggers, it consistently elicits an immune response that plays a key role in determining the severity and progression of the disease. Since the Interleukin-1 (IL-1) cytokine family is a key player in the innate immune response during pancreatitis, this study aims to investigate the role of IL-1 α , a member of the IL-1 family, in the course of acute and chronic pancreatitis. Specifically, we seek to understand the impact of IL-1 α knockout on disease severity. Till now nothing is known about the function and role of IL-1 α during acute and chronic pancreatitis. While IL-1 β and the inflammasome complex is well investigated in various mouse models, not much is known about the impact of IL-1 α on pancreatic diseases. In contrast to IL-1 β which is mainly released by macrophages and monocytes, IL-1 α is widely expressed in various cell types. In the first step we want to identify the source of IL-1 α during pancreatitis. We want to identify the cells that secrete it, its site of action, the responding cell types, and its mechanism of action.

The second aim is to investigate the role of IL-1 α on disease severity of acute and chronic pancreatitis by using IL-1 α deficient mice. Here we want to investigate the impact of IL-1 α on local and systemic inflammation and pancreatic damage during acute form of pancreatitis induced by caerulein. Furthermore, we want to investigate the effect of IL-1 α on organ remodeling and fibrogenesis in a mouse model of chronic pancreatitis.

Finally, we want to test the hypothesis that IL-1 α is a therapeutic target for the treatment of acute and chronic pancreatitis. Therefore, we want to use the IL-1R1 antagonist in a mouse model of acute and chronic pancreatitis to block the IL-1 receptor signaling induced by IL-1 α and IL-1 β . Here we want to investigate the immune response and pancreatic damage in a model of acute pancreatitis and the fibrogenesis in a model of chronic pancreatitis. At the time of hospitalization, the immune response is already activated, making it the most promising common therapeutic target for treating or preventing severe disease progression.

2. Material and Methods

2.1. Material

2.1.1. Laboratory equipment and consumables

Laboratory equipment and consumables	Manufacturer
1 ml syringes	B. Braun (Melsungen, Deutschland)
6-well plates	Falcon (Amsterdam, Niederlande)
12-well plates	Grainer bio-one (Frickenhausen, Deutschland)
24-well plates	Falcon (Amsterdam, Niederlande)
96-well-plates U-bottom	Grainer bio-one (Frickenhausen, Deutschland)
96-well-plates V-bottom	Falcon (Amsterdam, Niederlande)
384 well plates	Biozym Scientific GmbH (Oldendorf, Deutschland)
Adventurer® balance Ara520	OHAUS Europe GmbH (Nänikon, Schweiz)
Amersham Protran Premium 0.45 NC	Cytiva (Marlborough, Massachusetts, USA)
BD Plastipak fine dispensing syringes	Becton Dickinson (Franklin Lakes, New Jersey, USA)
BD™ LSR II flow cytometer	BD Biosciences (Heidelberg, Deutschland)
BD Vacutainer® SST™ II Advance	Becton Dickinson (Franklin Lakes, New Jersey, USA)
BioPhotometer® 6131	Eppendorf AG (Hamburg, Deutschland)
Cannulas	B. Braun (Melsungen, Deutschland)
Centrifuge 5424 R	Eppendorf (Hamburg, Deutschland)
Centrifuge 5425 R	Eppendorf (Hamburg, Deutschland)
Centrifuge 5430 R	Eppendorf (Hamburg, Deutschland)

Centrifuge 5702 R	Eppendorf (Hamburg, Deutschland)
Centrifuge 5810 R	Eppendorf (Hamburg, Deutschland)
Centrifuge tubes 15 ml	SARSTEDT AG & Co. (Nümbrecht, Deutschland)
Centrifuge tubes 50 ml	Falcon (Amsterdam, Niederlande)
Cell counting device Casy®1 model TT	Roche Innovatis AG (Bielefeld, Deutschland)
Cell culture incubator	Binder (Tuttlingen, Deutschland)
Cell strainers (50 µm, 70 µm, 100 µm)	Grainer bio-one (Frickenhausen, Deutschland) Miltenyi Biotech (Bergisch Gladbach, Deutschland)
Cover slips	R. Langerbrink (Emmendingen, Deutschland)
Cryo-microtome	Leica (Wetzlar, Deutschland)
Cryotubes	Grainer bio-one (Frickenhausen, Deutschland)
Dounce Homogenisator	DWK Life Science (Wertheim am Main, Deutschland)
Electrophoresis	PeqLab (Erlangen, Deutschland)
Electrophoresis Power Supply – EPS 301	Amersham Biosciences (Amersham, United Kingdom)
Erlenmeyer flasks 10 ml and 25 ml	Lenz Laborglas (Wertheim, Deutschland)
FACS tubes	Sarstedt (Nümbrecht, Deutschland)
Filtropur S 0.45	SARSTEDT AG & Co. (Nümbecht, Deutschland)
Fine scale MC1 Analytic AC 120 S	Sartorius AG (Göttingen, Deutschland)
FluorChem SP	Alpha Innotech Cell Biosciences (San Leandro, Kalifornien, USA)
Fusion FX	Vilber Lourmat Deutschland GmbH (Eberhardzell, Deutschland)
Gauze compresses/gauze	Fuhrmann (Much, Deutschland)

GentleMACS C Tubes	Miltenyi Biotech (Bergisch Gladbach, Deutschland)
GentleMACS Dissociator	Miltenyi Biotech (Bergisch Gladbach, Deutschland)
Grant Bio™ PV-1 vortexer	Grant Instruments™ (Cambridge, United Kingdom)
Herasafe sterile workbench	Thermo Fisher Scientific Inc. (Waltham, USA)
Heating block	Eppendorf AG (Hamburg, Deutschland)
Ice machine	Manitowoc Company Inc. (Manitowoc, USA)
Mastercycler EP S Thermal Cycler	Eppendorf AG (Hamburg, Deutschland)
Micro hematocrit capillaries	BRAND (Wertheim, Deutschland)
Microplate reader SpectraMax 190	Molecular Devices (Sunnyvale, CA, USA)
Microscope	Zeiss (Oberkochen, Deutschland)
Microscope slide	R. Langerbrink (Emmendingen, Deutschland)
Microscope Olympus FLUOVIEW FV1000	Olympus (Hamburg, Deutschland)
Micro test tubes (0.5 ml, 1.5 ml, 2.0 ml)	Eppendorf AG (Hamburg, Deutschland)
Microtome	Leica (Wetzlar, Deutschland)
Microwave	Candy (Brugherio, Italien)
Millipore water treatment	Merck (Darmstadt, Deutschland)
Mini-PROTEAN® Tetra Cell	Bio-Rad (Hercules, Kalifornien, USA)
MSC-Advantage™ Biological Safety Cabinet	Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA)
PeqSTAR Thermal Cycler	PeqLab (Erlangen, Deutschland)
Petri dishes	SARSTEDT AG & Co. (Nümbrecht, Deutschland)
Photoimager	Alpha Innotech Cell Biosciences (San Leandro, CA, USA)
Photometer, SPECTRAmax Plus384	Molecular Devices (Sunnyvale, CA, USA)

Pipettes	Eppendorf AG (Hamburg, Deutschland)
Pipette tips 10 µl	Carl ROTH (Karlsruhe, Deutschland)
Pipette tips 200 µl	Grainer bio-one (Frickenhausen, Deutschland)
Pipette tips 1000 µl	Eppendorf AG (Hamburg, Deutschland)
PowerPac™ 200	Bio-Rad (Hercules, Kalifornien, USA)
Qiagen Tissue lyser	Qiagen (Hilden, Deutschland)
QuantStudio™ 7 Flex Real-Time PCR Instrument	Thermo Fisher Scientific Inc. (Waltham, USA)
Rotary shaker	UniEquip (Planegg, Deutschland)
Serological pipettes (5 mL, 10 mL, 25 mL)	SARSTEDT AG & Co. (Nümbrecht, Deutschland)
Shaking water bath 1092	LAUDA-GFL mbH (Burgwedel, Deutschland)
Shaking water bath GFL-1083	LAUDA-GFL mbH (Burgwedel, Deutschland)
Slide Scanner	Sysmex Corporation (Kōbe, Präfektur Hyōgo, Japan)
SpectraMax Plus384 microplate reader	Molecular Devices (Sunnyvale, Kalifornien, USA)
Sprout microcentrifuge	Heathrow Scientific (Vernon Hills, USA)
Tissue-Tec	Sakura (Staufen, Deutschland)
Tissue-Tek® Cryomold® cryoembedding cassettes 10 mm × 10 mm × 5 mm	Sakura Finetek Europe B.V. (Alphen aan den Rijn, Niederlande)
Trans-Blot™ SD Semi-Dry Transfer Cell	Bio-Rad (Hercules, Kalifornien, USA)
Universal 320 centrifuge	Andreas Hettich GmbH & Co. KG (Tuttlingen, Deutschland)
Vortexer MS2 mini shaker	IKA (Staufen, Deutschland)
VWR® Ergonomic High-Performance multi-channel pipette, 20-200 µl	VWR (Radnor, Pennsylvania, USA)
Whatman 3MM CHR Cellulose Chromatography Paper	Cytiva (Marlborough, Massachusetts, USA)

2.1.2. Chemicals/Kits

Chemicals/Kits	Manufacturer
10 × Target Retrieval Solution	Agilent Dako (Santa Clara, Kalifornien, USA)
Acetic acid	CHEMSOLUTE™, TH. Geyer GmbH & CO. KG (Renningen, Deutschland)
Acrylamide 30 %	Carl Roth GmbH (Karlsruhe, Deutschland)
Agarose	Carl Roth GmbH (Karlsruhe, Deutschland)
Amylase Kit	Roch (Basel, Schweiz)
Anakinra	Swedish Orphan Biovitrum (Sobi) (Stockholm, Sweden)
Azan dyeing kit	Morphisto GmbH (Frankfurt a.M., Deutschland)
APS	Carl Roth GmbH (Karlsruhe, Deutschland)
Bovines Serum albumin (BSA)	PAN-Biotech GmbH (Aidenbach, Deutschland)
Bradford-Kit	BioRad (Hercules, CA, USA)
Bromophenol Blue	Merck KGaA (Darmstadt, Deutschland)
Caerulein	Bachem Holding AG (Bubendorf, Schweiz)
CASYton	OMNI Life Science GmbH & Co KG (Bremen, Deutschland)
CBA Mouse Inflammation Kit	BD Biosciences (Heidelberg, Deutschland)
Cell Counting Kit-8 (CCK-8)	GLP BIO (Montclair, Kalifornien, USA)
Cholecystokinin (CCK) Fragment 26-33 Amide, non-sulfated	Sigma-Aldrich (St. Louis, Missouri, USA)
Collagenase	Serva Electrophoresis GmbH (Heidelberg, Deutschland)
Cryocompound Clear	Immunologic a WellMed company (Arnhem, Niederlande)
Dipotassium hydrogen phosphate	Merck KGaA (Darmstadt, Deutschland)
Disodium hydrogen phosphate	Merck KGaA (Darmstadt, Deutschland)

dNTP mix	Thermo Fisher Scientific Inc. (Waltham, USA)
DTT (0,1 M)	Invitrogen (Karlsruhe, Deutschland)
DMEM, high glucose, GlutaMAX™ Supplement	Gibco™, Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA)
EDTA	Carl Roth GmbH (Karlsruhe, Deutschland)
EDTA·2Na	Sigma (Taufkirchen, Deutschland)
Eosin	Sigma (Taufkirchen, Deutschland)
Ethanol	J.T. Baker (Deventer, Netherlands)
Ethidiumbromide	Carl Roth GmbH (Karlsruhe, Deutschland)
Fc receptor block	Miltenyi Biotech (Bergisch Gladbach, Deutschland)
Fetal calf serum (FCS)	GIBCO®, Invitrogen (Karlsruhe, Deutschland)
First-Strand Buffer (5x)	Invitrogen (Karlsruhe, Deutschland)
Fluorescence mounting medium	Agilent Dako (Santa Clara, USA)
Formaldehyde 4,5 %; pH7	UniApotheke (Greifswald, Deutschland)
Gelatine	Carl Roth GmbH (Karlsruhe, Deutschland)
Glycerin	Carl Roth GmbH (Karlsruhe, Deutschland)
Glycerol	Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA)
Glycin	Carl Roth GmbH (Karlsruhe, Deutschland)
Hydrogen peroxide (H ₂ O ₂)	Merck KGaA (Darmstadt, Deutschland)
Hämatoxylin	MORPHISTO GmbH (Offenbach am Main, Deutschland)
HEPES	Sigma-Aldrich (St. Louis, Missouri, USA)
High-capacity cDNA reverse transcription kit	Thermo Fisher Scientific Inc. (Waltham, USA)
HPLC-Wasser	J.T. Baker (Deventer, Niederlande)
Hydrogen peroxide (H ₂ O ₂)	Merck KGaA (Darmstadt, Deutschland)

Isopropanol	CHEMSOLUTE™, TH. Geyer GmbH & CO. KG (Renningen, Deutschland)
KCl	Merck KGaA (Darmstadt, Deutschland)
KH ₂ PO ₄	Merck KGaA (Darmstadt, Deutschland)
KHCO ₃	Sigma (Taufkirchen, Deutschland)
LIPC Kit	Roch (Basel, Schweiz)
Methanol	J.T. Baker (Deventer, Niederlande)
Multi Tissue Dissociation kit 3	Miltenyi Biotech (Bergisch Gladbach, Deutschland)
M-MLV Reverse Transcriptase (200 U/μL)	Invitrogen (Karlsruhe, Deutschland)
NaCl	Carl Roth GmbH (Karlsruhe, Deutschland)
Na ₂ HPO ₄ ·H ₂ O	Merck KGaA (Darmstadt, Deutschland)
NH ₄ Cl	Carl Roth GmbH (Karlsruhe, Deutschland)
O-dianisidine	Sigma (Taufkirchen, Deutschland)
Oligo dT	Invitrogen (Karlsruhe, Deutschland)
PageRuler™ Prestained Protein Ladder	Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA)
PCR Master Mix (2 ×)	Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA)
Penicillin-Streptomycin (10.000 U/ml)	Gibco™, Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA)
Phenylmethylsulfonylfluoride (PMSF)	Sigma (Taufkirchen, Deutschland)
Random Primer	Invitrogen (Karlsruhe, Deutschland)
RNaseOUT™ Recombinant Ribonuclease Inhibitor (40 U/μL)	Promega (Madison, USA)
Sevofluran	Abbott Laboratories (Chicago, USA)
Sodium azide	Merck KGaA (Darmstadt, Deutschland)
SuperSignal™ West Femto Chemiluminescence Kit	Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA)
SuperSignal™ West Pico Chemiluminescence Kit	Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA)

SYBR™ green PCR master mix	Thermo Fisher Scientific Inc. (Waltham, USA)
TEMED	Sigma-Aldrich (St. Louis, Missouri, USA)
Tris	Carl Roth GmbH (Karlsruhe, Deutschland)
Triton X-100	Ferak Berlin GmbH (Berlin, Deutschland)
Trizol	Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA)
VectaMount™ Permanent Mounting Medium	Vector Laboratories (Newark, Kalifornien, USA)
Xylene cyanol FF	Merck KGaA (Darmstadt, Deutschland)
Xylol	Carl Roth GmbH (Karlsruhe, Deutschland)
β-Mercaptoethanol	Merck KGaA (Darmstadt, Deutschland)

2.1.3. Buffers and solutions

Buffers/Solutions	Composition	
Acinar cell isolation medium	DMEM	90 ml
	Hepes (100mM, without pH adjustment)	10 ml
	BSA (5 %)	23 ml
	HCL (1M)	1 – 1.5 ml
Agarose gel	1x TAE-Buffer	
	Agarose	1 % - 2 %
	Ethidium bromide	1.78 µmol/l
Blocking solution (immunofluorescence)	PBS	
	FCS	20 %
Cell culture medium	DMEM or RPMI	450 ml
	Fetal calf serum (FCS)	50 ml
	Penicillin/streptomycin	5 ml
DNA loading buffer	H2O	
	Tris-HCl (pH 7.6)	10 mM
	Bromphenol Blue	0.03 %

	Xylene cyanol FF	0.03 %
	Glycerol	60 %
	EDTA	60 mM
	H ₂ O	10 mM
Erythrocyte lysis buffer (10x)	H ₂ O	
	NH ₄ Cl	1.5 M
	KHCO ₃	0.1 M
	EDTA·2Na	10 mM
FACS buffer	PBS	
	Sodium azide	0.02 %
	FCS	2 %
	EDTA	2 mM
Laemmli Puffer 2x	TRIS-HCL pH 6.8	125 mmol/l
	SDS	4 %
	Glycerin	30 %
	Bromophenol blue	0.01 %
	β-mercaptoethanol in H ₂ O	10 %
MPO extraction buffer	H ₂ O	
	KH ₂ PO ₄	50 mM
	Hexacetyltrimethylammonium bromide	0.5 %
	pH 6.0 (adjusted with KOH)	
MPO homogenization buffer	H ₂ O	
	KH ₂ PO ₄	20 mM
	pH 7.4 (adjusted with KOH)	
MPO measurement buffer	H ₂ O	
	KH ₂ PO ₄	50 mM
	pH 6.0 (adjusted with KOH)	
	O-dianisidine	0.53 mM
	H ₂ O ₂	0.21 %
	NaCl	150 mmol/l

NET-Gelatin Block Buffer Western Blot	EDTA	5 mmol/l
	TRIS-HCl	50 mmol/l
	Triton X-100	0.05 %
	Gelatine in H ₂ O	0,02 %
NET buffer Western blot	NaCl	150 mmol/l
	EDTA	5 mmol/l
	TRIS-HCl	50 mmol/l
	Triton X-100 in H ₂ O	0.05 %
PBS (10 x)	H ₂ O	
	NaCl	1.37 M
	KCl	27 mM
	KH ₂ PO ₄	15 mM
	Na ₂ HPO ₄ ·H ₂ O	81 mM
	pH 7.4	
Permeabilization solution (Immunofluorescence)	PBS	
	FCS	20 %
	Triton-X	0.1 %
Running buffer SDS-PAGE	TRIS	25 mmol/l
	Glycin	192 mmol/l
	SDS in H ₂ O	0.01 %
TAE (10 x)	Acetic acid	0.2 M
	H ₂ O	
	Tris	0.4 M
	EDTA (pH 8.0)	10 mM
Towbin Transfer buffer Western Blot	Tris	3.02 g
	Glycin	14.4 g
	SDS (10 %)	10 %
	Methanol in H ₂ O (1 l)	20 %
	pH 8.3	

2.1.4 Oligonucleotide

The oligonucleotides used were synthesized by Invitrogen

Primer	Forward 5' → 3'	Reverse 5' → 3'
<i>5s</i>	GCCCGATCTCGTCTGATCTC	GCCTACAGCACCCGGTATTC
<i>Ccl2</i>	AGTAGGCTGGAGAGCTACAA	GTATGTCTGGACCCATTCCTTC
<i>Col1a1</i>	CAGACTGGCAACCTCAAGAA	CAAGGGTGCTGTAGGTGAAG
<i>Col3a1</i>	ATGGCTCACCAGGACAAAG	CACCAGGACTGCCGTTATT
<i>Fn1</i>	GCCTGAGGTGGACCCCGCTA	GGGCCCAAGTGACCCGCATC
<i>Il1a</i>	TCTCAGATTCACAACCTGTTCTGTG	AGAAAATGAGGTCCGGTCTCACTA
<i>Il6</i>	CCAGAGTCCTTCAGAGAGATACA	CCTTCTGTGACTCCAGCTTATC
<i>Il1b</i>	GAGGACATGAGCACCTTCTTT	GCCTGTAGTGCAAGTTGTCTAA
<i>Il1r1</i>	ACGGGTTTAGTTCGGAGCTG	TCATATTCTCCTGGGCGTGC
<i>Il1racp</i>	GAATTTGTGCTGCTGACGCTG	TGGAGCACGTAGTTGGGACTT
<i>Acta2</i>	GCCAGTCGCTGTCAGGAACCC	CCAGCGAAGCCGGCCTTACA

2.1.5 Primary antibodies

Antibody	Species	Manufacturer
4',6-Diamidine-2' phenylindole dihydrochloride (DAPI)	-	Sigma (Taufkirchen, Deutschland)
Anti-Amylase (sc-46657)	Mouse	Santa Cruz (Dallas, TX, USA)
Anti-CCR2	Rabbit	Abcam (Cambridge, UK)
Anti-CD206 (OASA05048)	Rat	Aviva Systems Biology (San Diego, CA, USA)
Anti-CD68 (ABIN181836)	Rat	Antibodies-online (Aachen, Deutschland)
Anti-Collagen (ab34710)	Rabbit	Abcam (Cambridge, UK)
Anti-FAP	Rabbit	Abcam (Cambridge, UK)
Anti-FGFR (D8E4)	Rabbit	Cell Signaling (Danvers, USA)

Anti-GAPDH	Mouse	Meridian Bioscience (Cincinnati, Ohio, USA)
Anti-IL-1r1	Rabbit	Abcam (Cambridge, UK)
Anti-IL-1 α	Rabbit	Sino Biological (Düsseldorf, Deutschland)
Anti-Ki67	Rat	Bethyl Laboratories (Montgomery, USA)
Anti-Nfkbp65	Rabbit	Cell Signaling (Danvers, USA)
Anti- α SMA (1A4)	Mouse	Agilent DAKO (Santa Clara, USA)

2.1.6 Secondary antibodies

Antibody	Manufacturer
Anti-Mouse IgG-Alexa 488-Conjugated	Jackson Immun Research (West Grove, PA, USA)
Anti-Mouse IgG-Cy5-Conjugated	Jackson Immun Research (West Grove, PA, USA)
Anti-Rabbit IgG-Cy3-Conjugated	Jackson Immun Research (West Grove, PA, USA)
Anti-Rabbit IgG-Cy5-Conjugated	Jackson Immun Research (West Grove, PA, USA)
Anti-Rat IgG-Cy3-Conjugated	Jackson Immun Research (West Grove, PA, USA)
Anti-Rat IgG-Cy5-Conjugated	Jackson Immun Research (West Grove, PA, USA)
Anti-Mouse IgG-HRP-Conjugated	Amersham plc (Amersham, United Kingdom)
Anti-Rabbit IgG-HRP-Conjugated	Amersham plc (Amersham, United Kingdom)

2.1.7 Antibodies for flow cytometry

Specificity	Conjugate	Clone	Manufacturer	Final concentration
αSMA	AF488	1A4	Invitrogen	10 µg/mL
CCR3	APC/Cy7	J073E5	BioLegend®	4 µg/mL
CD11b	PerCP/Cy5.5	M1/70	BioLegend®	4 µg/mL
CD34	PECy5	MEC14.7	BioLegend®	4 µg/mL
CX3CR1	PE/Cy7	SA011F11	BioLegend®	4 µg/mL
GFAP	AF647	2.2B10	Invitrogen	4 µg/mL
IL-1R1b	BV421	4E2	BioLegend®	4 µg/mL
IL-4Rα	PE	I015F8	BioLegend®	4 µg/mL
PDGFRa	BV605	APA5	BioLegend®	4 µg/mL

2.1.8 Software

Software	Manufacturer
Alpha Innotech	Alpha Innotech Cell Biosciences (San Leandro, Kalifornien, USA)
CaseViewer 2.4	Sysmex GmbH (Norderstedt, Deutschland)
CellSens	Olympus (Hamburg, Deutschland)
FlowJo_V10	BD Biosciences (Heidelberg, Deutschland)
Fusion Capt Advance	Vilber Lourmat Deutschland GmbH (Eberhardzell, Deutschland)
GraphPad Prism 8.01	GraphPad Software, Inc. (La Jolla, Kalifornien, USA)
Microsoft Excel 2019	Microsoft Corporation (Redmond, USA)
Microsoft PowerPoint 2019	Microsoft Corporation (Redmond, USA)
Microsoft Word 2019	Microsoft Corporation (Redmond, USA)
Quant Studio™ 6 and 7 Flex	Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA)
SlideViewer 2.5	Sysmex GmbH (Norderstedt, Deutschland)

Soft MaxPro 6.3	Molecular Devices (Sunnyvale, Kalifornien, USA)
Zotero 6.0.15	Corporation for Digital Scholarship (Vienna, Virginia, USA)

2.2 Methods

2.2.1 Animal experimental methods

2.2.1.1 Animal husbandry

Male and female C57BL/6 and IL-1 α knockout mice (C57BL/6 genetic background), aged 8 to 16 weeks and weighing 20 to 30 g, were used in the experiments. Breeding pairs of IL-1 α deficient mice (C57BL/6J-II1a^{em1Tdk}/J) were purchase from JAX[®] Mice & Services Wild type animals were purchased from Charles River. C57BL/6J-II1a^{em1Tdk}/J were bred at the Central Service and Research Facility for Laboratory Animals (ZSFV) at the University of Greifswald. The experiments were conducted in the biotechnical facility or the laboratories of research clusters III or IIIa, in accordance with the German Animal Welfare Act (LALLF 7221.3-1-035/22).

2.2.1.2 Pancreatitis models

Pancreatitis was induced by the intraperitoneal injection of caerulein model to induce both acute and chronic pancreatitis in mice. Animals were fasted for 8–12 hours before onset of the disease, with free access to water. Acute pancreatitis was triggered by hourly intraperitoneal injections of caerulein (50 μ g/kg body weight) for up to 8 hours, a schematic representation of the animal model of acute pancreatitis is shown in Figure 4. Chronic pancreatitis was induced by administering the same dose for up to 6 hours per day, 3 days a week, over 4 weeks, Figure 5. Caerulein, an analogue of the hormone cholecystokinin (CCK), induces pancreatitis by overstimulating pancreatic acinar cells, leading to premature protease activation. To reduce injection-related pain, analgesic medication (Tramal[®] at 1 mg/ml) was added to the drinking water, beginning 24 hours prior to and continuing through the experiment. Animals were sacrificed at various intervals throughout the experiment. Anakinra (10mg/kg/body weight) was administered i.p. at the time points outlined in Figure 4 and 5.

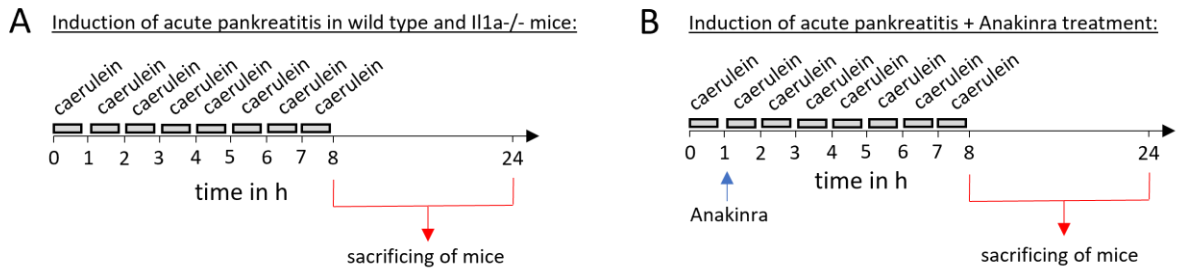


Figure 4: Treatment regimen for acute pancreatitis. (A) For wild type and IL-1 α ^{-/-} mice, and (B) for treatment with anakinra.

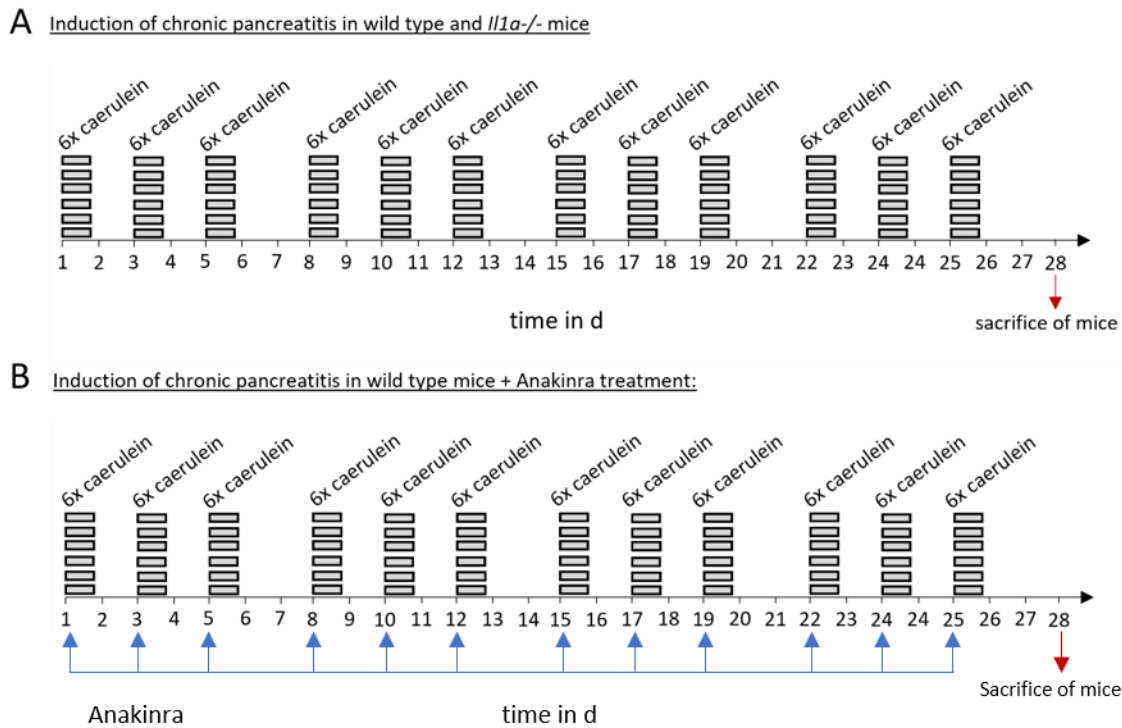


Figure 5: Treatment regimen for chronic pancreatitis. (A) For wild type and IL-1 α ^{-/-} mice, and (B) For treatment with anakinra.

2.2.1.3 Sacrificing animals and removal of organs

Depending on the time point, the animals were anesthetized with sevoflurane, and blood was collected in serum tubes retro-orbitally. The blood was then centrifuged (5 min at 5000 rpm) in a serum tube, and the resulting serum was stored at -80°C for subsequent analysis.

After blood collection, the animals were sacrificed by cervical dislocation. The pancreas, spleen, lungs, and lymph nodes were extracted and either shock-frozen in liquid nitrogen and stored at -80°C or fixed in 4% formaldehyde for paraffin embedding and histological analysis. Alternatively, pancreatic tissue was embedded in Cryo-compound Clear, shock-frozen in liquid nitrogen, and stored at -80°C. The paraffin embedding process was performed by staff at the Institute of Pathology at the University Medical Center Greifswald.

Additionally, the spleen and blood were collected for flow cytometric and FACS analysis.

2.2.2. Flow cytometry and FACS

2.2.2.1 Cell staining and flow cytometry

Cells in 100 μ L blood samples were stained for flow cytometric analyses in a FACS tube. For this, the cells were first washed with FACS buffer (1200 rpm, 6 min) and the cell pellet was then dissolved with 1 μ L Fc block in 25 μ L FACS buffer and incubated for 5 min at 4 °C. 25 μ L of the antibody mixture (in FACS buffer) were then added for extracellular staining (depending on the FACS panel, see Table 1 (incubation 30 min, 4 °C). For intracellular staining, the cells first had to be permeabilized (Transcription Factor Staining kit, Miltenyi Biotec; see manufacturer's instructions). After permeabilization, incubation was carried out again with 1 μ L Fc-Block (in 25 μ L FACS buffer, 5 min, 4 °C) and with 25 μ L antibody mixture (in FACS buffer) for intracellular staining (depending on the FACS panel, see Table 1) (30 min, 4 °C). After the staining steps, the cells were resuspended in 150 μ L FACS buffer, analyzed in the flow cytometer (BD, LSRII) and evaluated with FlowJo.

Table 1: Fibroblasts panel

Antibody	Fluorochrome	Dilution
Anti-IL-1R1b (extracellular)	BV421	1:50
Anti-IL-4R α (extracellular)	PE	1:50
Anti-CD34 (extracellular)	PECy5	1:50
Anti-PDGFR α (extracellular)	BV605	1:50
Anti-CX3CR1 (extracellular)	PE/Cy7	1:50
Anti-CD11b (extracellular)	PerCP/Cy5.5	1:50
Anti-CCR3 (extracellular)	APC/Cy7	1:50
Anti-GFAP (extracellular)	AF647	1:50
Anti- α SMA (extracellular)	AF488	1:50

2.2.3 Histological staining

1-2 μ m sections of paraffin embedded tissue samples were cut by microtome. Cryosections were cut by cryotome at -20°C of 1-2 μ m thickness. Cryosections were fixed with ice cold acetone at -20°C for 20 min, and stored at -20°.

2.2.3.1 Hematoxylin-eosin staining

Hematoxylin-eosin staining is used in histology to stain various structures of a tissue. Hematoxylin stains all basophilic structures blue like the cell nuclei. Eosin is a synthetic acidic dye and stains all acidophilic structures red and these are mainly the cytoplasmic proteins.

Paraffin sections were deparaffinized by incubating in xylol for 10 minutes, followed by methanol for 5 minutes, then xylol for another 10 minutes, and methanol for 5 minutes. This was followed by treatment with a descending alcohol series (100%, 95%, 70%), with 5 minutes for each concentration, and a final wash in PBS for 5 minutes.

The sections were stained with hematoxylin for 5 minutes and eosin for 1 minute, followed by an ascending alcohol series (70%, 95%, 100%) for a few seconds each. Finally, they were incubated in xylol for 5 minutes and embedded in VectaMount Permanent Mounting Medium. Scoring of pancreatic damage was performed according to Niederau C. (74).

2.2.3.2 Azan blue staining

Using Azan staining the cells nuclei, collagen structures and cytoplasm can be differentiated. The red dye azocarmine is used to stain the nuclei and the blue dye aniline is used to stain connective tissue structures.

The staining was carried out on paraffin embedded tissues according to the manufacturer's instructions (staining kit: Azan according to Heidenhain, MORPHISTO GmbH) and at the end the tissues were mounted with VectaMount.

2.2.3.3 Immunofluorescence labeling

Immunofluorescence labeling is a technique used to identify proteins or other structures using antibodies tagged with fluorescent dyes. For paraffin sections, the initial steps involved deparaffinization using xylol and methanol, followed by treatment with decreasing concentrations of alcohol and then PBS. Antigen retrieval was performed by boiling the sections in DACO antigen retrieval solution for 30 minutes and subsequently cooled in PBS. After three PBS washes, the sections were blocked with 20% FCS in PBS for 1 hour.

For cryo-sections, frozen slides fixed with 20% acetone were used. The sections were blocked with 20% FCS/PBS for 1 hour at 4 °C. The primary antibody, diluted in 20% FCS at

concentrations of 1:100, 1:200, or 1:500, was applied to the sections and incubated overnight at 4 °C. After three PBS washes, a fluorescently labeled secondary antibody was added and incubated for 1 hour at room temperature. Following three additional PBS washes, DAPI staining was performed, and the sections were mounted in Fluorescence Mounting Medium (Dako) and stored at 4 °C until analysis.

2.2.3.4 Scanning and quantification

All stained slides were scanned using a slide scanner and analyzed with a fluorescent microscope. Quantification of fibrotic areas and cells positive for specific markers was performed using the CellQuant tool in CaseViewer software.

2.2.4 Myeloperoxidase determination of the lung

Myeloperoxidase is an enzyme found in neutrophils and monocytes, playing a significant role in inflammatory responses and serving as a marker for leukocyte infiltration. For tissue homogenization, lung samples stored at -80°C were combined with 500 µL of homogenization buffer and ground using a Douncer. A 100 µL portion of the tissue suspension was taken for protein measurement, treated with ultrasound for two 10-second intervals, and centrifuged at 10,000 g at 4 °C. The remaining 400 µL were similarly centrifuged (10,000 g, 4 °C), with the supernatant discarded and the pellet resuspended in 500 µL of extraction buffer. Each sample underwent four cycles of rapid freezing in liquid nitrogen and thawing at 37 °C. Subsequently the samples were sonicated twice for 10 seconds and centrifuged for 10 minutes at 10,000 g at 4°C. The supernatant, designated for MPO analysis, was stored at -80°C in a 1 to 5 dilution in measurement buffer. For the enzyme assay, 90 µL of a substrate solution (0.167 mg/mL o-dianisidine, 0.21% H₂O₂) was mixed with 10 µL of diluted sample. Photometric measurements at 460 nm were taken immediately over 10 minutes at 30 °C, using a standard of purified myeloperoxidase for comparison. The linear increase in enzyme activity was analyzed and normalized to the respective protein content.

2.2.5 Determination of serum amylase and lipase

The enzyme activity of lipase and amylase was measured in the serum of the mice as a marker for the severity of the disease using the photometric Amyl2 and LipC kits from Roche-Hitachi according to the manufacturer's instructions. The serum was diluted 1:50 in PBS beforehand.

10 µl of diluted samples were then mixed with 90 µl of the respective substrate solution. The activity was measured photometrically in triplicates at 37 °C for 30 min at 405 nm for amylase and at 570 nm for lipase. The linear increase in enzyme activity was used for the evaluation.

2.2.6 Determination of cytokine concentration in serum

The concentration measurement of various cytokines in the serum of the animals was carried out as a FACS analysis using fluorescent antibody-coupled beads. Customized panel of LEGENDplex™ kit was used according to the manufacturer's instructions.

2.2.7 Molecular biological methods

2.2.7.1 RNA Isolation

To extract the total RNA, the respective organs were first homogenized using the TissueLyser II. To do this, the tissue was treated directly in a 2 ml Eppendorf tube with 500-1000 µL TRIzol™ and then chopped by adding stainless steel balls in the TissueLyser II. The cell suspension was then mixed with 200 µL chloroform (per 1 mL TRIzol™) and incubated for 2 min at RT. After centrifugation (14000 rpm, 15 min, 4 °C), the cell suspension was separated into three phases: the upper colorless phase contains the RNA; the middle phase is called the interphase and contains the DNA; and the lower phase is the phenol-chloroform phase. The upper phase was removed and pipetted into a new 2 mL tube. The addition of 500 µL isopropanol led to precipitation of the RNA (incubation for 10 min at RT). After centrifugation (14000 rpm, 10 min, 4 °C), the resulting RNA pellet was washed with 1 mL 75% ethanol (per 1 mL TRIzol™). Finally, the pellet was dried at RT and, depending on its size, dissolved in 30 - 100 µL RNase-free water, incubated for 10 min at 55 °C and overnight at 4 °C. The respective RNA concentration was measured using an Eppendorf BioPhotometer® 6131.

2.2.7.2 cDNA synthesis

For gene expression analyses using quantitative real-time PCR, the RNA must be transcribed into complementary, single-stranded DNA (cDNA). This reverse transcription was carried out using RNA-dependent DNA polymerase, random hexamers and oligo-dT primers. The reaction was carried out on the Eppendorf Mastercycler ep Gradient 5341. The substances used and the temperature profile used are shown in Table 2.

Table 2: Components and temperature profile of cDNA synthesis

Substance	Volume per batch	Amount
total RNA in A. dest	RNA+H ₂ O= 9.5 µL	2 µg of RNA
dNTP mix (10 mM)	1 µL	0.5 mM
Oligo dT (100 µM)	1 µL	5 µM
Random Primer (3 µg/µL)	0.5 µL	75 ng
Step	Temperature	Time
Primer annealing	65 °C	5 min
Cooling	4 °C	1 min
Substance	Volume per batch	Amount
First-Strand Buffer (5 x)	4 µL	1 x
DTT (0.1 M)	2 µL	0.01 M
RNaseOUT™ Recombinant Ribonuclease inhibitor (40 U/µL)	1 µL	40 U
M-MLV reverse transcriptase (200 U/µL)	1 µL	200 U
Step	Temperature	Time
Primer annealing	25 °C	10 min
Reverse transcription	37 °C	90 min
Enzyme inactivation	70 °C	15 min
Cooling	4 °C	1 min

The synthesized cDNA had a final concentration of 100 ng/µl.

2.2.7.3 Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) is used to amplify DNA fragments of specific DNA sequences. Based on repeated temperature gradients, the original DNA is denatured, primers bind to the single strand and the DNA is at the end enzymatically replicated. Thermostable DNA polymerase is used for this. The substances used and their composition are shown in Table 3. The reaction was carried out on the Peqlab Thermal Cycler.

Table 3: Composition of the PCR reaction mixture

Substance	Volume	Amount
Primer Mix= Forward (10 μ M) + Reverse (10 μ M) primers	0.3 μ L	0.15 μ M
Thermo-scientific PCR Mastermix (2x)	5 μ L	-
A. dest	3.7 μ L	-

Table 4: Temperature-time program of the thermal cycler

Step	Temperature	Time	Cycles
Initial denaturation	94 °C	3 min	
Denaturation	95 °C	45 sec	30 x
Annealing	54 °C	45 sec	
Elongation	72 °C	45 sec	
Final Elongation	72 °C	5 min	
Cooling	4 °C	Infinity	

2.2.7.4 Quantitative Real-time PCR

The quantification of gene expression of target genes can be investigated using quantitative real-time PCR. This method is based on sequence-specific amplification of the cDNA in real time. A dye (SYBR® Green) that is used intercalates into double-stranded DNA. The intensity of SYBR® Green increases after each PCR cycle and is detected in the amplification plot. The fluorescence intensity of the binding to double-stranded DNA is stronger than the binding to single-stranded DNA. Specific primers are used to detect desired DNA fragments. The Ct value marks the PCR cycle when the fluorescence signal becomes distinguishable from the background.

To carry out quantitative real-time PCR, SYBR® Green PCR Master Mix (2 x), 100 nM of the respective primers (reverse and forward) and 1 μ L cDNA (1:10 dilution) were used in duplicate (5 μ L per well). An NTC control containing water instead of cDNA was used to exclude contamination.

Table 5: Composition of the real Time PCR reaction mixture

Substance	Volume per batch
SYBR (x 2)	2.5 μ L
Forward primer (10 μ M)	0.15 μ L
Reverse primer (10 μ M)	0.15 μ L
Nuclease free water	1.2 μ L
cDNA (1/10 diluted)	1 μ L
Total sum	5 μ L

The Fast-Real-Time PCR System 7900HT (Applied Biosystems) was used according to the standard temperature profile, the respective annealing temperature was adjusted.

The data were evaluated using the $2^{-\Delta\Delta C_t}$ method. The mean C_t values of the duplicate determination were subtracted from the mean C_t values of the housekeeping gene 5S (ΔC_t value). The ΔC_t value of untreated control animals was again subtracted to normalize to the basal expression ($\Delta\Delta C_t$ value). Finally, the relative gene expression was determined using the $2^{-\Delta\Delta C_t}$ calculation.

2.2.8 Protein biochemical methods

2.2.8.1 Protein determination

The protein concentration was quantitatively determined using the Bradford assay. In this method, the Coomassie Brilliant Blue dye in the Bradford reagent forms a complex with the proteins in the sample, causing a shift in the absorption maximum from 470 nm to 590 nm, which can be measured with a photometer. To calculate the concentration, a calibration curve was first generated using BSA. The analysis was conducted with a SpectraMax Plus384 microplate reader and Soft MaxPro 6.3 software from Molecular Devices.

2.2.8.2 SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to separate the samples based on molecular mass. The gel was prepared in advance (Table 6), beginning with pouring a separating gel, followed by a stacking gel layered on top.

Table 6: Recipes for the SDS-PAGE gels used.

Substance	Resolving gel 15%	Stacking gel
A. dest	2.3 ml	1.4 ml
Acrylamide (30%)	5 ml	0.33 ml
1 mol/l Tris (pH 8.8)	2.5 ml	-
1 mol/l Tris (pH 6.8)	-	0.25 ml
SDS (10 %)	0.1 ml	0.02 ml
APS (10 %)	0.1 ml	0.02 ml
TEMED	0.004 ml	0.002 ml

The samples were mixed in equal parts with 2× Laemmli buffer and then boiled at 95 °C for 5 minutes. Once cooled, the samples were loaded onto the gel. The PageRuler™ Prestained Protein Ladder was used as a molecular weight marker.

The Mini-PROTEAN® Tetra Cell from Bio-Rad was used as the SDS chamber, with a Tris-Glycine-SDS buffer as the running buffer. Electrophoresis was conducted at 80 V until the lowest marker bands nearly reached the end of the gel. The gel was then ready for further analysis.

2.2.8.3 Western Blot

The Western blot is used for the antibody-based detection of a protein after prior separation according to its molecular weight using SDS-PAGE. The proteins in the SDS gel were transferred to a nitrocellulose membrane using the semi-dry method. The blotting buffer used was 1 × Towbin buffer, the Trans-Blot™ SD Semi-Dry Transfer Cell from Bio-Rad as the blotting chamber, the 3 mm CHR paper from Cytiva as the filter paper and the Amersham Protran Premium 0.45 µm or 0.2 µm nc from Cytiva as the membrane. The transfer was carried out at 10 V for 90 min. To check the transfer of the proteins, the membrane was then stained with Ponceau S staining solution for 10 sec and destained under running water. After checking the efficiency, the membrane was further destained under running water until all dye was removed. The membrane was then blocked using the blocking buffer (0.2% gelatin in 1 × NET buffer) for 1 h at room temperature on a shaker. Finally, the membrane was incubated overnight at 4 °C on a shaker with the primary antibody solution (1:1000 in blocking

buffer). The next day, the membrane was washed six times for 5 min each in 1 × NET buffer and then incubated with the secondary antibody (1:16000 in blocking buffer) for 1 h at room temperature on a shaker. The membrane was then washed six times with 1 × NET buffer. Development was carried out using the SuperSignal™ West Femto Chemiluminescence Kit or SuperSignal™ West Pico Chemiluminescence Kit from Thermo Scientific. The enhancer solution and the substrate solution were mixed together in a 1:1 ratio and evenly applied to the membrane. The membrane was then evaluated using the Fusion FX ChemoCam from Vilber Lourmat and the associated software. The substrate is converted by the secondary antibody-coupled peroxidase, which leads to a black precipitate at the level of the protein on the membrane.

2.2.9 Primary cell methods

2.2.9.1 Isolation of acinar cells

Live murine acinar cells were isolated through collagenase digestion. To begin, the pancreas was extracted from the mouse and placed in 6 ml of acinar cell medium containing 0.5 mg of collagenase. The tissue was finely chopped with scissors for approximately 1 minute. The resulting suspension was incubated at 37 °C in a water bath set at 100 rpm for 15 minutes. Fresh medium with collagenase was then added, and the tissue was chopped again for 1 minute. Following another 15-minute incubation at 37 °C, the suspension was pipetted up and down 10-20 times, using a pipette with a pre-cut tip. This process was repeated five times, with the last resuspension conducted without cutting the tip. The cell suspension was filtered through a double-layer gauze pad and centrifuged at 1800 rpm for 1 minute at room temperature. The supernatant was carefully removed, and 6 ml of fresh acinar cell medium was added. Filtration through gauze was repeated once more. Afterward, 15 ml of fresh medium was added, and the tube was placed horizontally in a water bath at 37 °C and 50 rpm for 30 min. A portion of the isolated acinar cells was stimulated with CCK (1:1000) and incubated in a water bath at 50 rpm for 30 minutes. Both stimulated and unstimulated acinar cell suspensions were then centrifuged at 1800 rpm for 1.5 minutes. The supernatant was discarded, and the cell pellet was collected for RNA isolation prior to the qPCR experiment.

2.2.9.2 Isolation of BMDM

Bone marrow-derived macrophages were isolated from the femur and tibia of mice under sterile conditions. The bone marrow was flushed from the bones using PBS and passed through a 70 µm cell strainer. The cell suspension was centrifuged for 10 minutes at 800 rpm and 4°C. The resulting pellet was resuspended in RPMI medium (supplemented with 10% FCS and 1% PenStrep). The cells were seeded into culture plates, and after 4 hours of incubation, the medium was replaced with fresh RPMI, and M-CSF (diluted 1:1000) was added to each plate. The medium was changed every two days, and the cells were used for experiments 5-7 days after isolation.

2.2.9.3 Isolation of pancreatic fibroblasts

Pancreatic fibroblasts were isolated using the Multi Tissue Dissociation Kit 3. The pancreas was shaken with 900 µl of buffer X and 100 µl of enzyme T for 10 minutes at 37°C and 140 rpm. Following this, the tissue suspension was transferred to 3 ml of DMEM (supplemented with 10% FCS and 1% P/S) and dissociated using the GentleMACS Dissociator under rotation (52 seconds, 287 runs). The resulting cell suspension was passed through a 40 µm cell strainer, centrifuged (2300 rpm for 2:30 minutes), and the pellet was resuspended in 10 ml of media. FGF2 (diluted 1:1000) was then added to the resuspended cells. The medium was changed every two days, and the cells were used for experiments 5-7 days after isolation. The cells were used for 3 purposes as illustrated below.

2.2.9.3.1 Immunofluorescence staining of isolated cells

The isolated fibroblasts were seeded in 8-well chamber slides and incubated for 24 hours. They were then stimulated with different cytokines under four conditions: control, IL-1α (20 ng/mL), TGF-β (10 ng/mL), and co-stimulation with IL-1α (20 ng/mL) + TGF-β (10 ng/mL). After 24 hours of stimulation, the cells were fixed with acetone at -20°C for 20 minutes and stained with specific markers following the protocol described in section 2.2.3.3. The slides were then analyzed using a fluorescence microscope.

2.2.9.3.2 RNA isolation and qPCR

Same stimulation conditions for the isolated fibroblasts as in 2.2.9.3.1 were used. After 24 hours of stimulation, RNA isolation was performed, followed by qPCR to assess the expression of genes encoding various proteins.

2.2.10 Transcriptome and proteome analysis

Pancreatic fibroblasts and BMDM were stimulated with 10 ng/mL IL-1 α cytokine for 24 hours. RNA was isolated using TRIzol, with the RNA phase used for gene expression analysis via the Affymetrix GeneChip Array and the protein-containing phase for proteomic analysis. Gene expression analysis was performed by the Functional Genomics Department. Proteome analysis was conducted by Leif Steil and Elke Hamer, and IPA pathway analysis by Sabine Ameling, all within the Functional Genomics Department. In silico pathway and functional analysis of differentially expressed genes was carried out using the commercial, systems biology-oriented software Ingenuity Pathway Analysis (Ingenuity Systems, Redwood, CA).

2.2.10.1 Affymetrix GeneChip Array

Differential gene expression analysis was performed on the isolated cells using the Affymetrix GeneChip Array, in collaboration with the Functional Genomics Department, to gain an overview of the genes encoding various proteins that are upregulated and downregulated in the experiment. IPA (Ingenuity Pathway Analysis) was also conducted on the Affymetrix Chip Array data to identify key biological pathways and networks associated with the differentially expressed genes.

2.2.10.2 Proteome analysis

Proteome analysis was performed on the isolated cells in collaboration with the Functional Genomics Department to provide an overview of the proteins that are upregulated and downregulated in the experiment.

2.2.11 Cell count determination

To determine the cell number, 10 μ l of a cell suspension were taken up in 10 ml CASYton buffer and then analyzed using the Casy[®] 1 Model TT and the 60 μ m capillary.

2.2.12 Cell proliferation assay of pancreatic fibroblasts

The isolated pancreatic fibroblasts were stimulated with 10 ng/ml of IL1 α cytokine in DMEM supplemented with 10 % FCS and 1 % penicillin/streptomycin for 24 hours. The proliferation of both stimulated and unstimulated cells was then assessed using the CCK-8 assay, following the protocol described below.

Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) from GLPBIO, which relies on the conversion of WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) into WST-8-formazan in the presence of 1-methoxyphenazine methyl sulfate. This reaction is proportional to the number of viable cells. The resulting WST-8-formazan, an orange dye, can be quantified photometrically at 450 nm. For the assay, 10000 cells per well were plated in a 96-well microtiter plate and incubated for 24 hours at 37 °C with 5% CO₂. A new microtiter plate was prepared for each measurement and time point. After 24 of cells stimulation under the experimental conditions, 10 µl of CCK-8 reagent was added to each well, followed by a 3-hour incubation at 37 °C with 5% CO₂, after which the absorbance was measured at 450 nm.

2.2.13 Statistical analysis

Excel (Microsoft Corporation, Redmond, USA) and GraphPad Prism 5.01 (GraphPad Software, Inc., La Jolla, USA) were used to calculate the data, create graphs and for statistical analysis. Flow cytometric analyses were analyzed using FlowJo V10 (BD Biosciences, Heidelberg, Germany). Student's t-test was used to evaluate significant differences between two groups. $p < 0.05$ was considered statistically significant.

3. Results

This study aims to investigate the role of the IL-1 α cytokine in the pathomechanism of acute and chronic pancreatitis, with a particular focus on its influence on disease severity and the local immune response. To this end, global IL-1 α knockout C57BL/6 mice were utilized to elucidate the cytokine's role in disease progression, including acinar cell necrosis, pancreatic inflammation, systemic inflammation in acute pancreatitis, and fibrosis development in chronic pancreatitis. Furthermore, a series of experiments were conducted to evaluate the therapeutic efficacy of Anakinra in mitigating the severity of pancreatitis.

3.1 IL-1 α : Origin, Site of Action, and Involved Cell Types

Several experiments were conducted to identify the cells that release IL-1 α during pancreatitis and those that express the IL-1R1 receptor on their surface, allowing IL-1 α to bind and activate specific signaling pathways within the cell.

A previous experiment conducted by our group demonstrated that the transcriptional profiles of cytokines and chemokines, expressed as fold changes relative to untreated controls, revealed a distinctly pro-inflammatory signature in bone marrow-derived macrophages (BMDMs) following co-incubation with acinar cells. Specifically, genes encoding key pro-inflammatory cytokines, including IL-6, IL-1 α , IL-1 β , TNF- α , and Tnfsf10 (TRAIL)—were upregulated in BMDMs after exposure to acini (Fig. 6a)

Acinar cells were isolated from the pancreas of wild-type mice and stimulated with a supramaximal concentration of CCK to mimic pancreatitis. IL-1 α gene expression was assessed by qPCR, revealing a significant upregulation in acinar cells after 1 and 2 hours of CCK stimulation (Fig. 6b).

Immunofluorescence labeling for α SMA (a fibroblast marker) and IL-1R1 (the IL-1 α receptor) showed a clear colocalization of both markers on the surface of the same cells in the pancreas of wild-type mice (Fig. 6c).

Flow cytometry analysis was performed on serum samples from mice with chronic pancreatitis. The initial gating was performed on live, single cells using forward and side scatter (FSC/SSC) profiles. CD11b⁺ cells were then gated, encompassing both monocytes and

fibroblasts. To distinguish these populations, monocytes were identified as CX3CR1⁺ cells, while fibroblasts were defined by α SMA expression. Further gating on PDGFR and GFAP allowed us to specifically isolate fibroblasts. Finally, the expression of IL-1R1 was assessed in both monocytes and fibroblasts, revealing that monocytes were negative for IL-1R1, while fibroblasts were positive (Fig. 6d).

Western blot analysis demonstrates IL-1 α protein expression in both unstimulated and CCK-stimulated acinar cells (Fig. 6e).

An interesting observation was that the IL-1R1 receptor was localized on the surface of cells, which were completely distinct from the cells labeled with CD206 (alternatively activated macrophages). This was evident in the immunofluorescence labeling of CD206 and IL-1R1-positive cells in the pancreas of wild-type mice with chronic pancreatitis (Fig. 6f).

These results demonstrate that, in addition to macrophages, acinar cells also serve as a source of IL-1 α . However, the receptor for IL-1 α (IL-1R1) is detected almost exclusively on fibroblasts. Immunostaining reveals clear co-localization of IL-1R1 with α -SMA, a fibroblast marker, but not with CD206, an M2 macrophage marker.

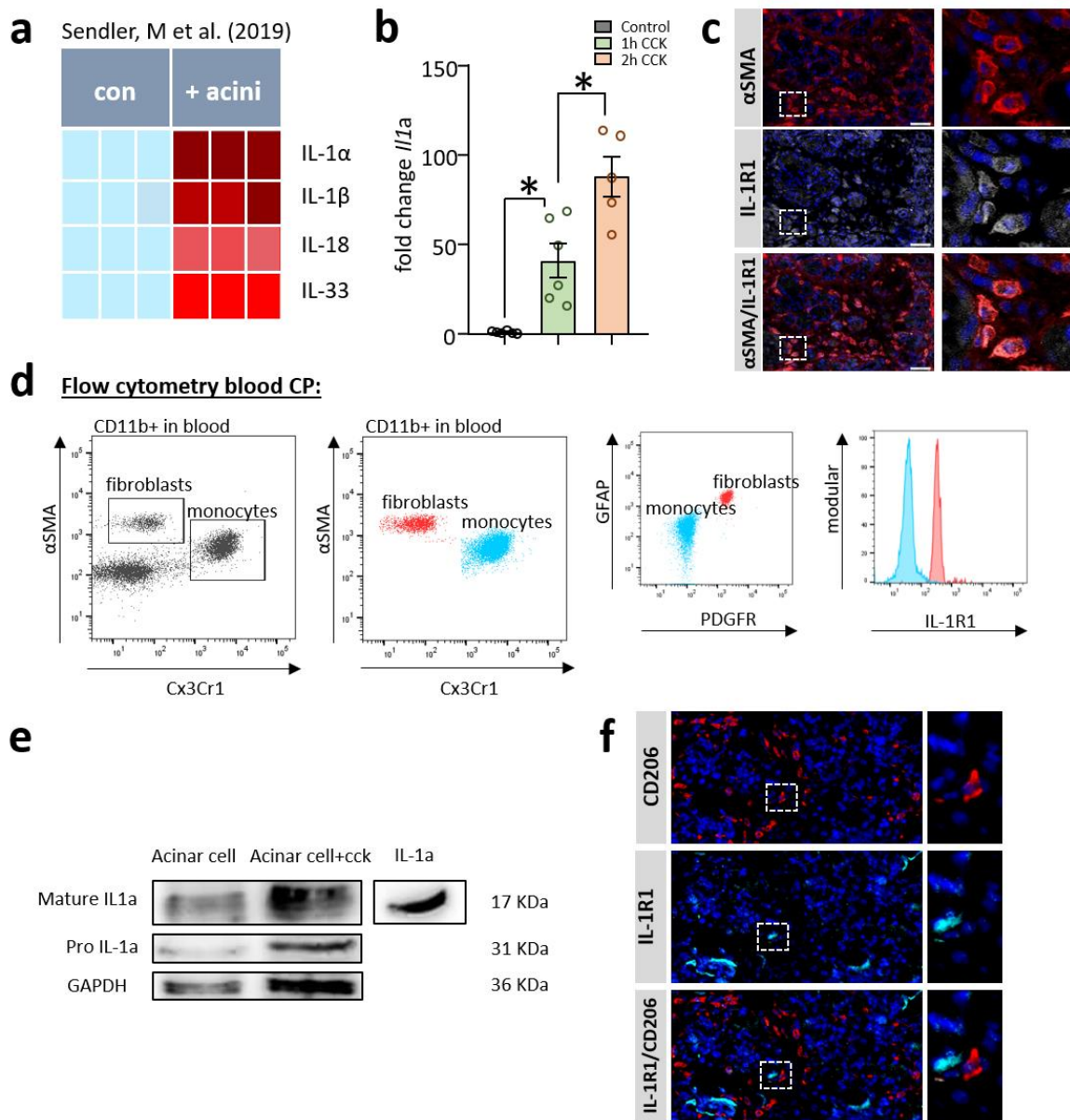


Figure 6: Investigating the source and site of action of IL-1 α .

(a) RNA chip array analysis from a previous experiment using bone marrow-derived macrophages (BMDMs) stimulated with DAMPs for 24 hours. (b) Quantitative PCR showing differential expression of *Il1a* after 1 and 2 hours of stimulating isolated pancreatic cells with CCK (Bar graph represents five biological independent samples). (c) Immunofluorescence staining of IL-1R1 and α -SMA in pancreatic tissue from mice with chronic pancreatitis (CP). (d) Flow cytometry analysis of serum from mice with CP (n=10). (e) Western blot analysis of IL-1 α expression in acinar cells with and without CCK stimulation (n=3 from each group). (f) Immunofluorescence staining of CD206 and IL-1R1 in pancreatic tissue from mice with CP. Statistical analysis was performed using an unpaired, two-sided *t*-test; $p < 0.05$ was considered statistically significant.

3.2 Impact of IL-1 α Knockout on the Severity of Acute Pancreatitis

Acute pancreatitis was induced in C57BL/6-J and IL-1 α ^{-/-} mice through repetitive hourly intraperitoneal injections of caerulein (50 μ g/kg/bodyweight) over an 8-hour period. Mice were sacrificed 8h and 24h after onset of disease, blood, pancreas, and lung tissues were collected for further analysis to determine whether IL-1 α influences disease severity or not.

Disease severity was assessed through histological analysis and serum enzymatic activity measurements. Paraffin-embedded pancreatic tissues were sectioned and stained with H&E for histopathological evaluation, including scoring of inflammation, necrosis, and edema. The pancreas of IL-1 α -deficient mice exhibited significantly reduced inflammatory cell infiltration, edema and acinar cell necrosis compared to C57BL/6 mice after 8 hours of acute pancreatitis induction (Fig. 7a). Blood samples were collected retro-orbitally into serum tubes, centrifuged (5 min at 5000 rpm), and the serum was isolated for enzymatic activity measurements of amylase and lipase. These enzyme activities act as markers for disease severity, as necrotic acinar cells release them into the bloodstream; higher serum enzyme activity indicates increased necrosis and therefore greater disease severity. The absence of IL-1 α led to a significant reduction in serum amylase and lipase activity compared to control mice, 8 hours after the induction of acute pancreatitis (Fig. 7b). It is noteworthy that 24 hours after pancreatitis induction, a significant reduction was observed in serum amylase and lipase levels (Fig. 7b), as well as in pancreatic damage—including edema and acinar cells necrosis (Fig. 7a)—in both wild-type and IL-1 α knockout mice. However, immune cell infiltration remained elevated 24 hours after disease induction (Fig. 7a).

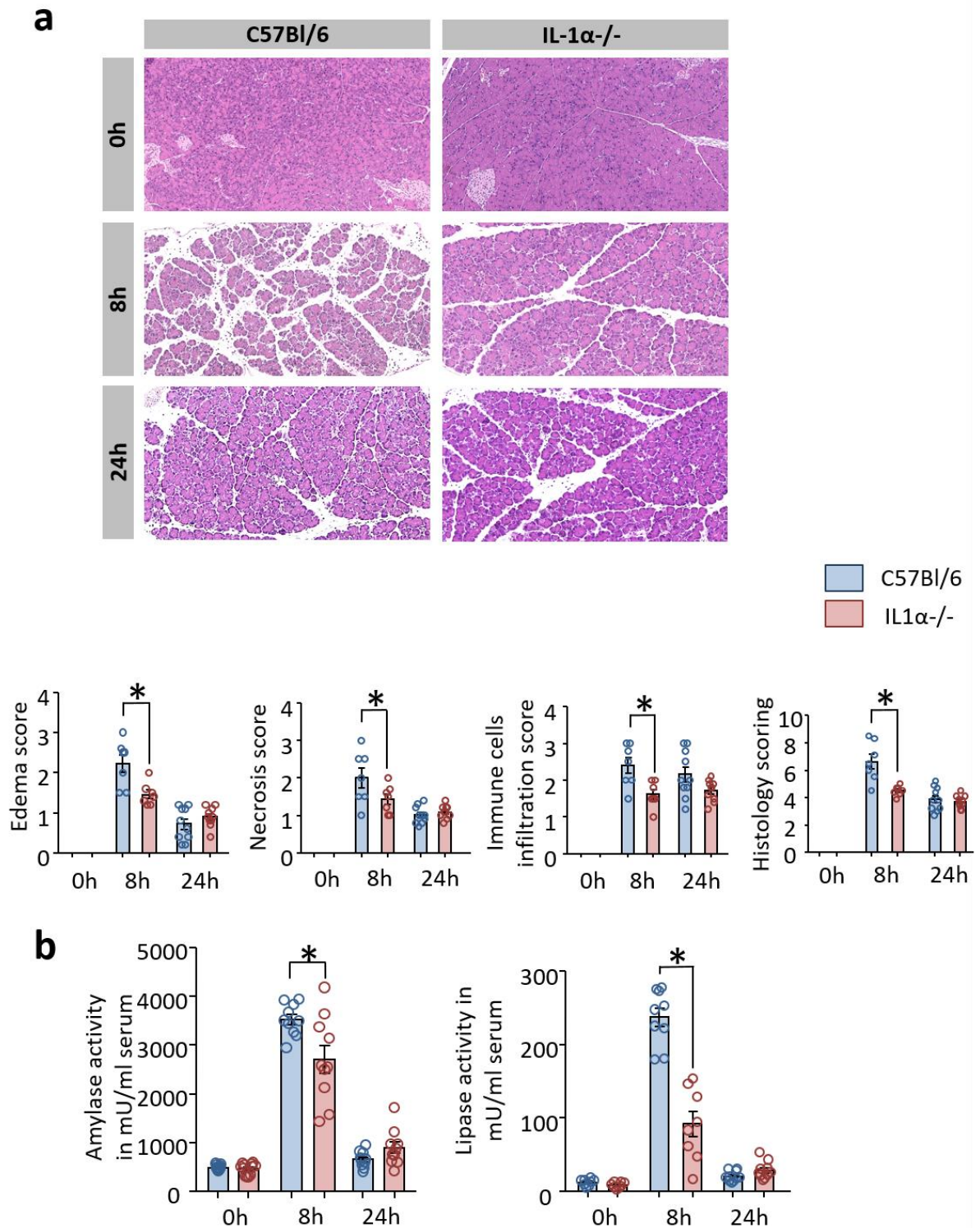


Figure 7: Assessment of acute pancreatitis severity in the absence of IL-1 α cytokine.

These experiments were conducted on both C57BL/6 wild-type and IL-1 α -/- mice induced with acute pancreatitis. **(a)** H&E staining of paraffin-embedded pancreatic sections, with scoring of inflammation, edema, and necrosis. **(b)** Measurement of amylase and lipase enzymatic activity in the serum. Statistical analysis was performed using an unpaired two-sided t-test, with significance set at * $p < 0.05$ ($n = 8-10$ per group at each time point).

The local inflammation of the pancreas was further evaluated using multiple methods, including immunofluorescence labeling of CCR2⁺ and CD68⁺ cells. CCR2 serves as a homing marker for infiltrating leukocytes in the pancreas, while CD68 is a general marker of macrophages, which is highly expressed on classical activated macrophages. Immunofluorescence analysis revealed a significantly lower infiltration of macrophages in the pancreas of IL-1 α -deficient mice compared to control mice with acute pancreatitis. Quantification of CD68⁺ and CCR2⁺ cells further confirmed a higher abundance of these macrophages in the pancreas of control mice than in IL-1 α ^{-/-} mice (Fig. 8a). Additionally, immunofluorescence labeling of Ki67⁺ and CD68⁺ cells was performed to assess macrophages proliferation in the pancreas. Ki67 is a marker of proliferating cells, while CD68, as mentioned earlier, identifies especially classically activated macrophages. This labeling demonstrated a significantly lower presence of Ki67⁺ CD68⁺ cells in the pancreas of IL-1 α -deficient mice compared to control mice with acute pancreatitis. The quantification of Ki67⁺ CD68⁺ cells further highlights this reduction (Fig. 8b).

Systemic inflammation was assessed by measuring cytokine levels in the serum of mice, which revealed a significantly reduced concentration of the pro-inflammatory cytokine IL-6, along with a decreased level of TNF α , in the absence of IL-1 α compared to wild-type mice with acute pancreatitis (Fig. 8c). Additionally, myeloperoxidase (MPO) activity in the lungs was measured, which reflects systemic immune cell mobilization. MPO activity showed a significant decrease in MPO activity in IL-1 α -deficient mice compared to wild-type mice with acute pancreatitis (Fig. 8d).

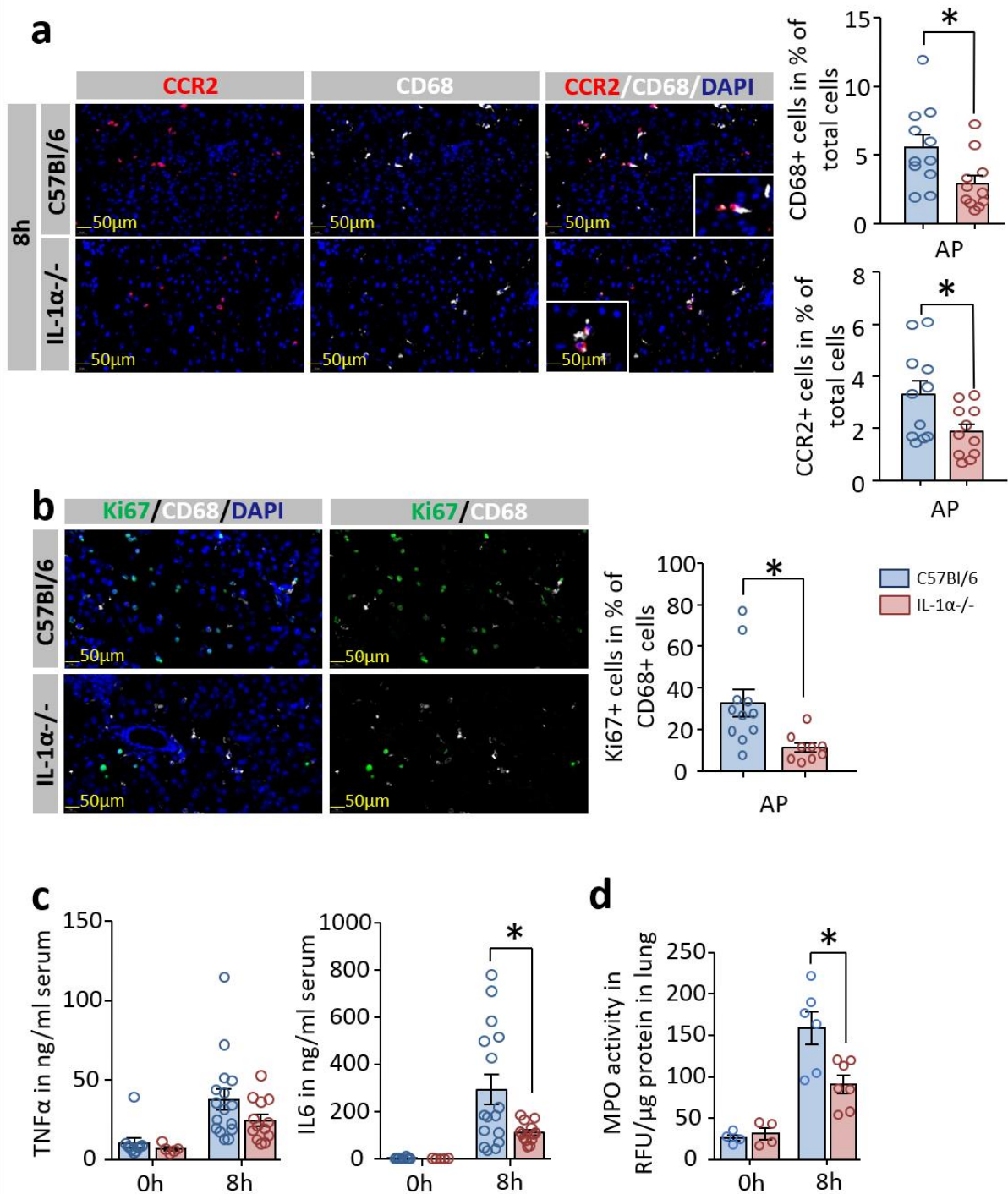


Figure 8: Assessment of acute pancreatitis severity in the absence of IL-1 α cytokine.

These experiments were conducted on both C57BL/6 wild-type and IL-1 α ^{-/-} mice induced with acute pancreatitis. **(a)** Immunofluorescence labeling of CCR2⁺ and CD68⁺ cells, with quantification of infiltrating macrophages in the pancreas 8h after onset of disease (n=11/group at 8h). **(b)** Immunofluorescence labeling of Ki67⁺ and CD68⁺ cells, with quantification of proliferating macrophages in the pancreas (n=11/group at 8h). **(c)** Serum cytokine measurement using a customized LegendPlex kit (n=6-10/group at 0h and n=16/group at 8h). **(d)** Myeloperoxidase enzymatic activity measurement in the lungs of mice (n=4-7/group at 0h and 8h). Statistical analysis was performed using an unpaired two-sided t-test, with significance set at *p < 0.05.

Our studies of acute pancreatitis in IL-1 α -deficient mice showed a clear reduction in disease severity in the absence of IL-1 α . One possible explanation is the reduced infiltration of immune cells into the pancreas, which contributes to acinar cell damage.

3.3 Blocking the IL-1R1 Pathway with Anakinra and Its Effect on Acute Pancreatitis Severity

To validate our previous findings, we investigated the impact of blocking the IL-1R1 pathway and preventing IL-1 α from binding to its receptor on the severity of acute pancreatitis by treating mice with intraperitoneal injections of the drug Anakinra. Mice induced with acute pancreatitis (following the protocol outlined in section 3.2) received a single anakinra injection (10 mg/kg body weight) one hour after the initial caerulein injection. The mice were sacrificed 8 and 24h after onset of pancreatitis, and disease severity was assessed by measuring serum amylase and lipase enzymatic activity, as well as evaluating necrosis, edema, and immune cell infiltration in the pancreas.

Histological analysis (H&E staining) further confirmed these findings. At the eight-hour mark, pancreata from anakinra-treated mice displayed reduced edema, acinar cell necrosis, and immune cell infiltration compared to untreated mice at the same disease stage. By 24 hours post-induction, both groups exhibited diminished edema and immune cell infiltration, with no evident differences between them (Fig. 9a).

Notably, mice treated with anakinra exhibited lower serum amylase and lipase activity levels eight hours after pancreatitis induction compared to untreated mice. However, by 24 hours post-induction, pancreata from both treated and untreated groups showed signs of recovery, with a marked reduction in serum enzymatic activity and no significant differences between the two groups (Fig. 9b).

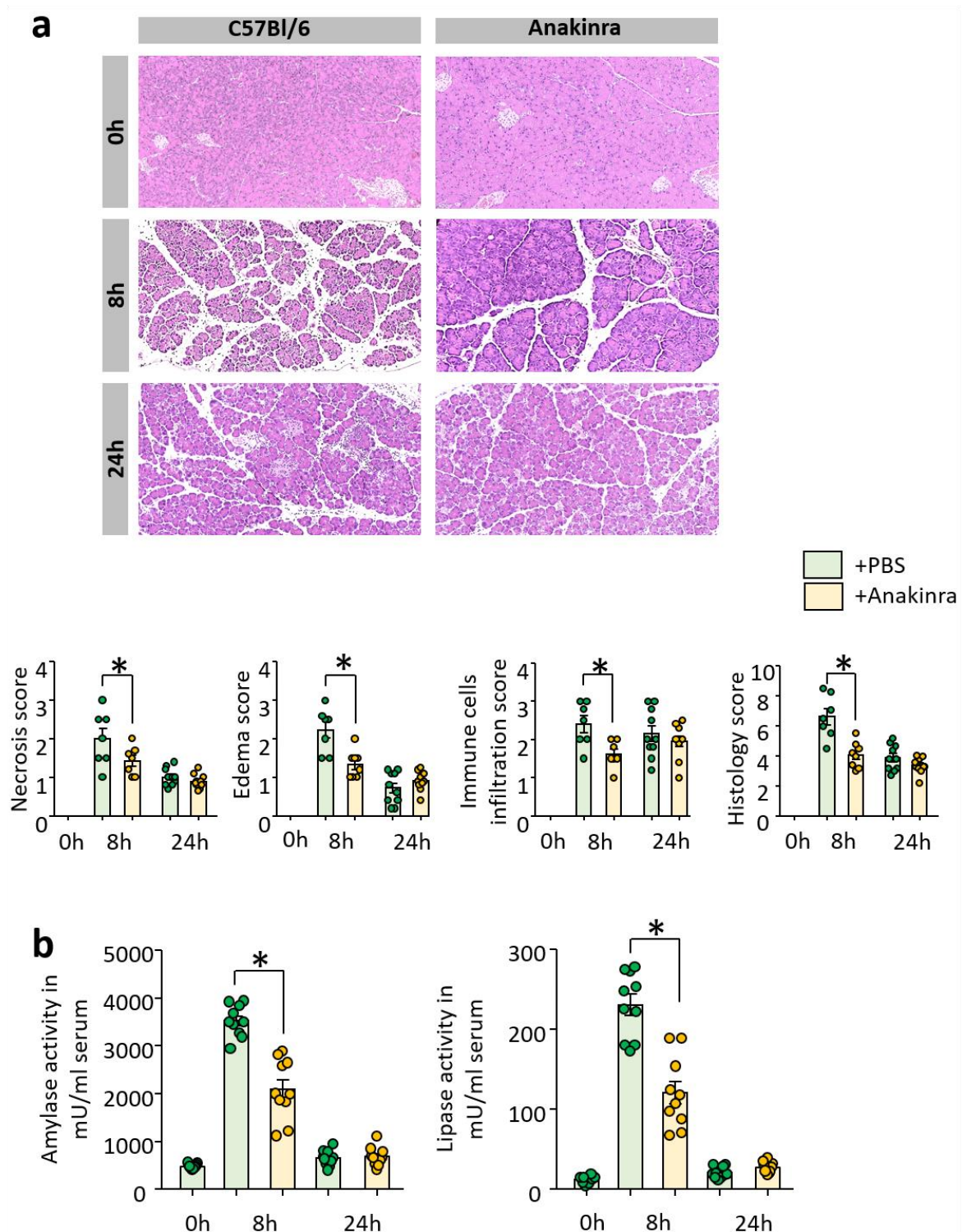


Figure 9: Assessment of Anakinra influence of acute pancreatitis severity.

(a) H&E staining of pancreatic tissue from untreated and Anakinra-treated wild-type mice at 0, 8, and 24 hours following acute pancreatitis induction. Histological scoring was performed to assess edema, acinar cell necrosis, and immune cell infiltration. **(b)** Measurement of serum amylase and lipase enzymatic activity in both groups at the same time points. Statistical analysis was conducted using an unpaired, two-sided *t*-test; $p < 0.05$ was considered statistically significant ($n=10$ /group at each time point).

3.4 The Effect of IL-1 α deficiency on Chronic Pancreatitis Severity

Our previous results have shown that the receptor for IL-1 α is mainly expressed on fibroblasts. Since fibroblasts play a crucial role in the course of chronic pancreatitis, we used a chronic disease model in the following experiments to evaluate the role of IL-1 α during chronic pancreatitis. Chronic pancreatitis was induced like previously described in C57BL/6-J and IL-1 α deficient mice. At the day 28, mice were sacrificed, and disease severity was assessed by measuring the fibrosis percentage and fibroblast presence in the pancreas.

Azan blue and H&E staining were used to visualize damage, fibrosis and organ remodeling in the tissue. These staining techniques, along with our quantification, clearly demonstrated that IL-1 α -deficient mice exhibited a lower percentage of pancreatic fibrosis compared to wild-type mice with chronic pancreatitis (Fig. 10a).

Immunofluorescence labeling of FGFR+ and FAP+ fibroblasts in the pancreas revealed a significant reduction in their percentage in IL-1 α -deficient mice compared to controls with chronic pancreatitis. Similarly, immunofluorescence labeling of collagen and α SMA, another marker to identify fibroblast, demonstrated decreased collagen deposition and a lower abundance of fibroblasts in the pancreas of IL-1 α -deficient mice (Fig. 10b).

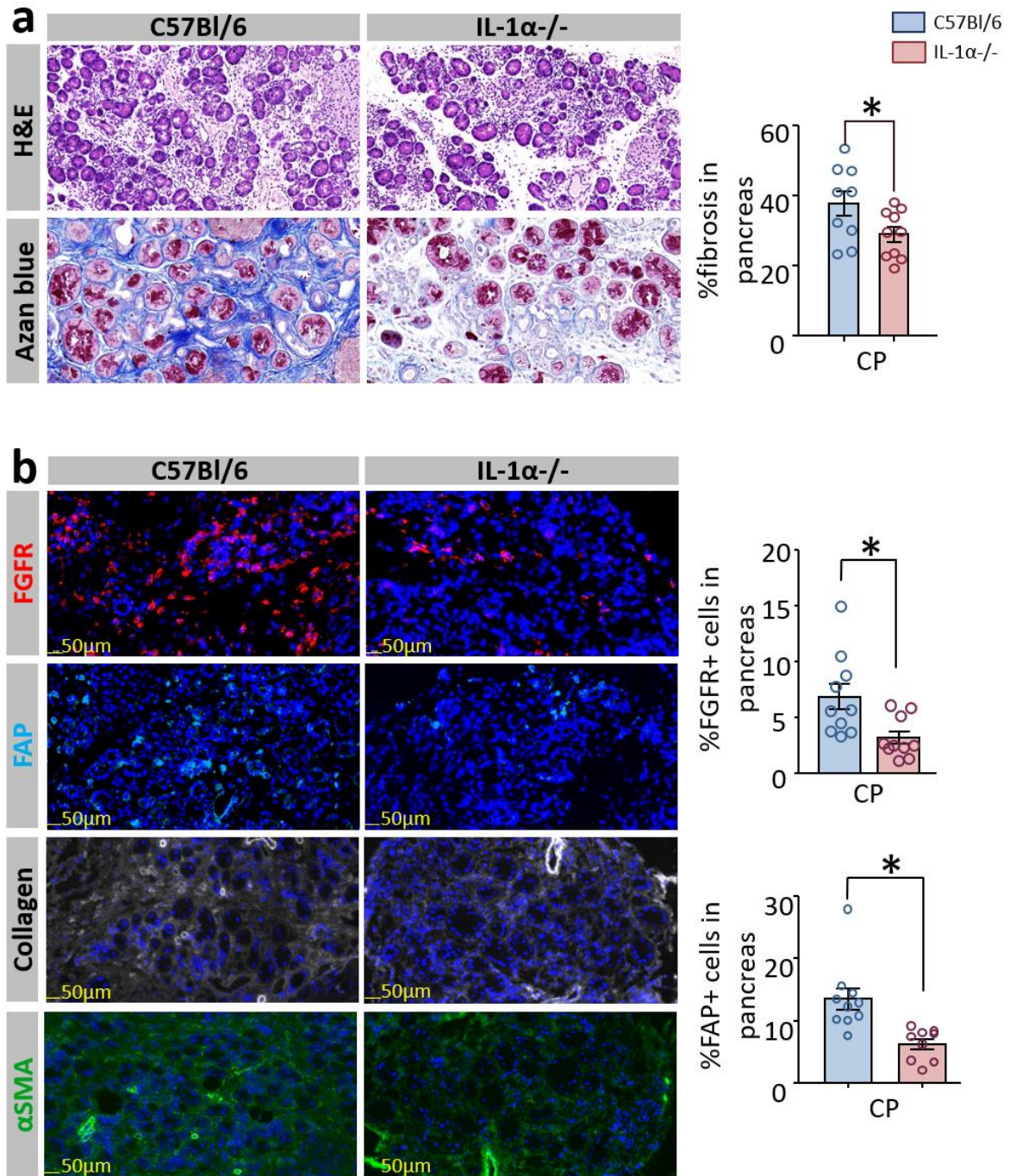


Figure 10: Evaluating the severity of chronic pancreatitis in the absence of IL-1 α cytokine.

These experiments were performed on C57Bl/6 wild-type and IL-1 α ^{-/-} mice induced with chronic pancreatitis. **(a)** H&E and Azan blue stainings to visualize pancreatic damage and quantify fibrosis. **(b)** Immunofluorescence labeling of FGFR+ FAP+ fibroblasts, collagen, and α SMA as markers of fibrosis, along with fibroblast quantification in the pancreas. Statistical analysis was conducted with an unpaired two-sided t-test, with significance set at * $p < 0.05$ ($n=10$ /group).

CD206+ cell labeling in the pancreas showed no significant change in the percentage of alternatively activated macrophages in IL-1 α -deficient mice compared to controls (Fig. 11a).

Ki67+ cell labeling revealed a reduced number of proliferating cells in the pancreas of IL-1 α -deficient mice compared to wild type mice with chronic pancreatitis. Amylase labeling indicated that most of these proliferating cells are acinar cells (Fig. 11b). Additionally, the labeling of Ki67+ cells and the α SMA marker in the pancreas showed not only fewer proliferating cells in the IL-1 α -deficient mice but also that these proliferating cells include pancreatic stellate cells along with acinar cells (Fig. 11c).

Measurement of serum cytokines using a customized LEGENDplex kit revealed a significant increase in IL-1 α concentrations in wild-type mice following the induction of chronic pancreatitis. In contrast, IL-1 β levels in the serum showed no significant change after chronic pancreatitis induction (Fig. 11d). Additionally, cytokine analysis showed that pro-inflammatory cytokines such as IL-6 and TNF α were slightly reduced in the absence of IL-1 α under chronic pancreatitis conditions. As expected, IL-1 α was completely absent in knockout

mice compared to controls, while TGF β levels showed no difference between IL-1 α ^{-/-} and control mice with chronic pancreatitis (Fig. 11e).

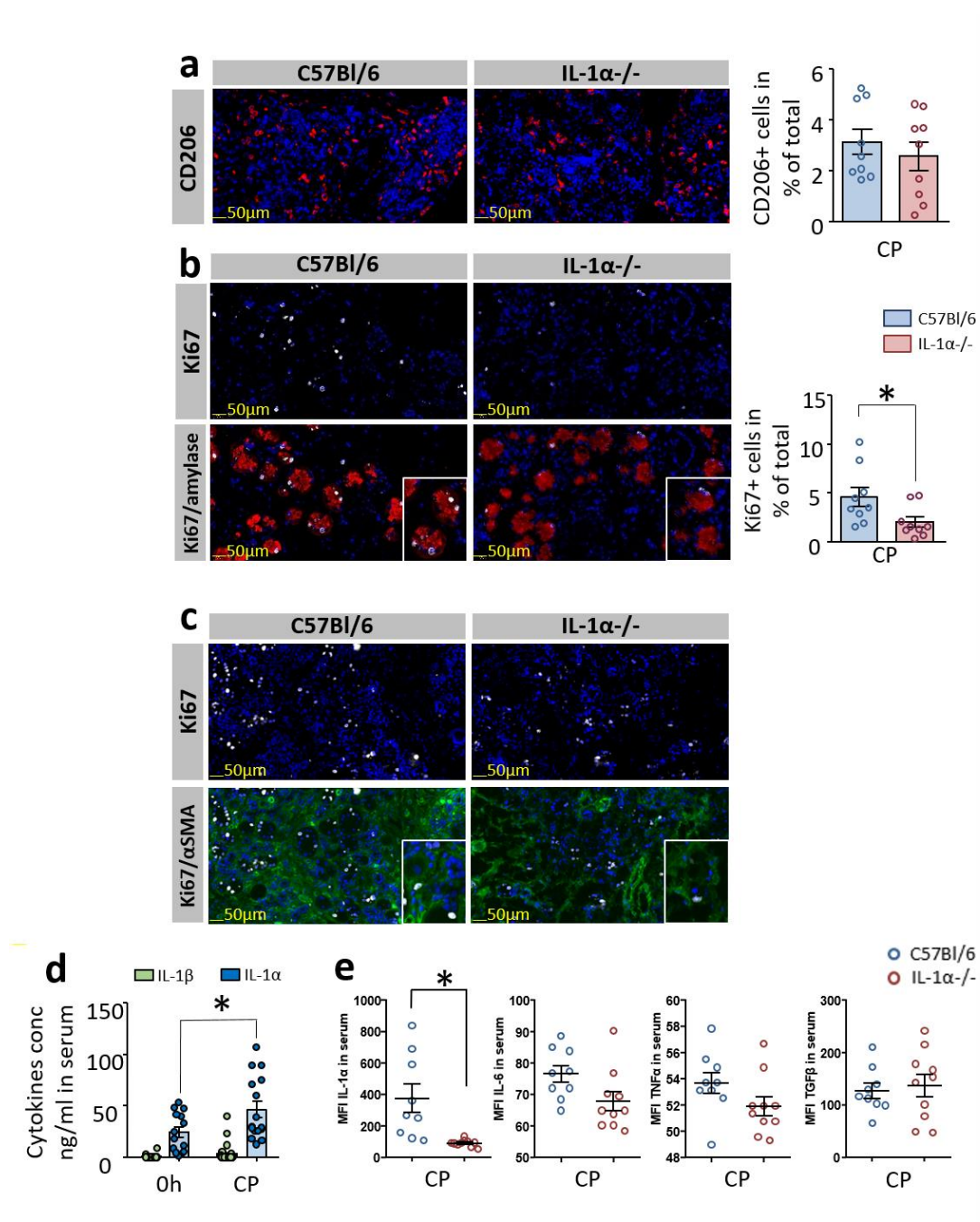


Figure 11: Assessing the severity of chronic pancreatitis in the absence of IL-1 α cytokine.

These experiments were performed on C57BL/6 wild-type and IL-1 α ^{-/-} mice induced with chronic pancreatitis. **(a)** Immunofluorescence labeling and quantification of CD206⁺ alternatively activated macrophages in the pancreas (n=9/group). **(b)** Ki67⁺ amylase⁺ proliferating acinar cell labeling and quantification of proliferating cells in the tissue (n=9/group). **(c)** Immunofluorescence labeling of Ki67⁺ α SMA⁺ proliferating fibroblasts in the pancreas. **(d) & (e)** Cytokine measurements in the serum of control and chronic pancreatitis mice using a customized LegendPlex kit (n=9-16/group). Statistical analysis was conducted with an unpaired two-sided t-test, with significance set at *p < 0.05.

These experiments demonstrated significantly reduced fibrosis during chronic pancreatitis in IL-1 α ^{-/-} mice compared to wild-type controls. Notably, while the number of pancreatic macrophages remained unchanged between groups, the number of fibroblasts was significantly lower in IL-1 α ^{-/-} mice.

3.5 Blocking the IL-1R1 Pathway with Anakinra and Its Effect on Chronic Pancreatitis Severity

In this step we were interested if blockage of the IL-1R1 signaling pathway could prevent pancreatic fibrogenesis. To examine the effect of IL-1R1 blockade on the severity of chronic pancreatitis, we induced the condition in C57BL/6 mice as described previously. In this experiment, however, the mice received intraperitoneal injections of Anakinra (10 mg/kg body weight) one hour prior to the first caerulein injection on each day of administration. This regimen was repeated three times per week for four weeks, resulting in a total of 12 Anakinra injections per mouse over the study period. The purpose of the Anakinra injections was to block IL-1R1, thereby inhibiting the binding of IL-1 α to this receptor. Essentially, we aimed to simulate the IL-1 α knockout (IL-1 α ^{-/-}) condition in the mice. At the day 28 of the experiment, the mice were sacrificed, and several analyses were performed to assess disease severity.

To evaluate pancreatic damage and fibrosis, H&E and Azan blue staining were conducted on pancreatic tissue from both Anakinra-treated and control mice. The staining demonstrated that the pancreata of Anakinra-treated mice exhibited a significantly lower percentage of fibrosis compared to the control group with chronic pancreatitis (Fig. 12a).

Furthermore, immunofluorescence labeling of fibrosis markers in the pancreas allowed quantification of fibroblasts and detection of differences between the two groups of mice. This analysis showed that FGFR⁺ FAP⁺ α SMA⁺ fibroblasts were significantly less abundant in the pancreata of Anakinra-treated mice compared to the untreated group with chronic pancreatitis (Fig. 12b).

Immunofluorescence labeling and quantification of CD206⁺ cells revealed no significant difference in the population of these cells between Anakinra-treated and untreated mice with chronic pancreatitis (Fig. 12c).

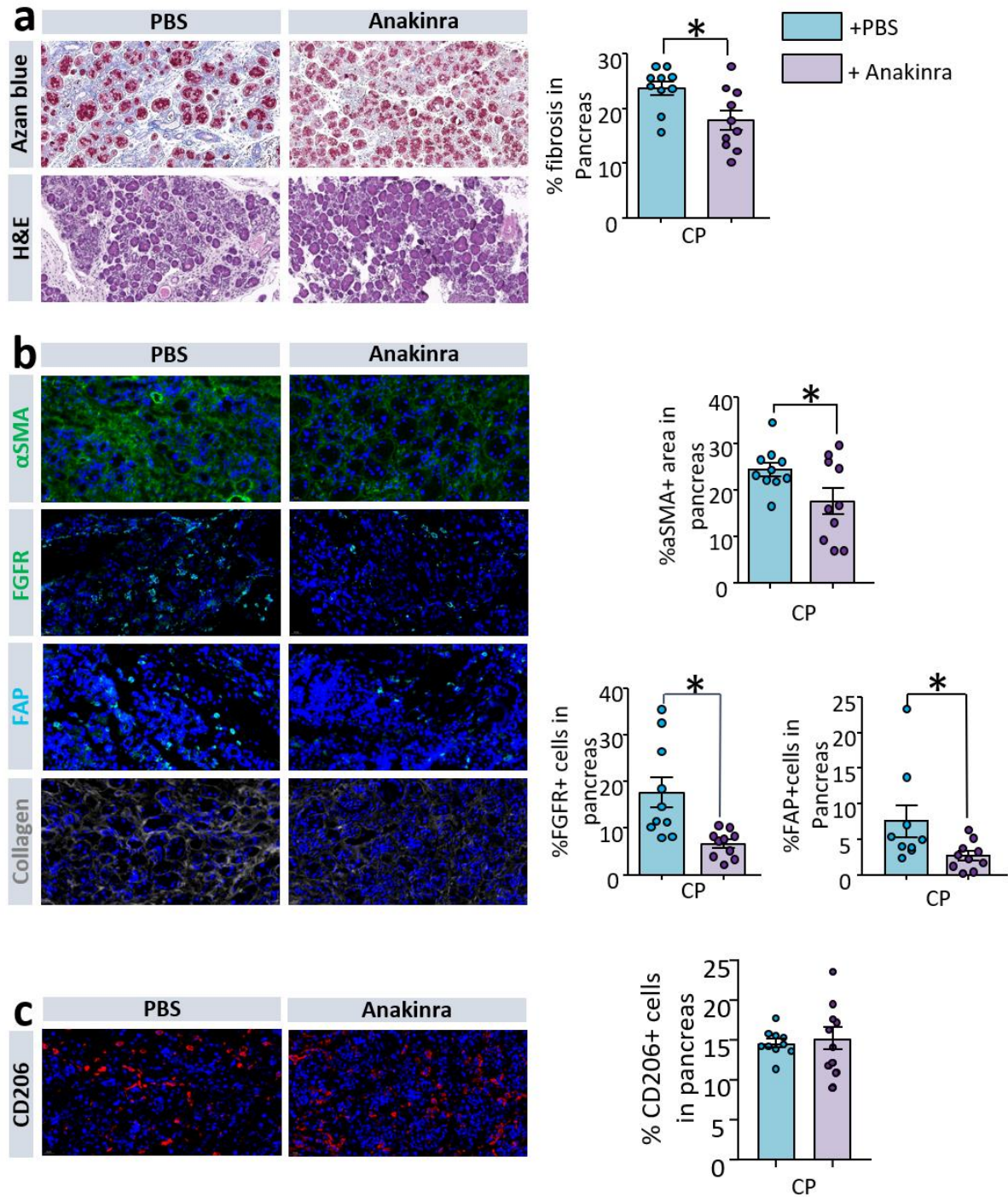


Figure 12: Assessment of Anakinra's Impact on Chronic Pancreatitis Severity.

All stainings and quantifications were performed on pancreatic tissue from chronic pancreatitis (CP) mice, both untreated and treated with Anakinra, to evaluate the therapeutic effects of the drug. **(a)** Histological analysis of pancreatic tissue using Azan blue and H&E staining, along with quantification of fibrosis levels. **(b)** Immunofluorescence staining for key fibrosis markers, including α SMA, FGFR, FAP, and collagen, to assess extracellular matrix remodeling. **(c)** Immunofluorescence labeling of CD206⁺ macrophages in pancreatic tissue. Statistical comparisons were performed using an unpaired, two-tailed t-test; differences with $p < 0.05$ were considered statistically significant ($n=10$ /group).

3.6 Influence of IL-1 α Stimulation on Pancreatic Fibroblasts and Macrophages: Transcriptomic, Proteomic, and Pathway Analysis

The following experiments were conducted to investigate cellular responses at both the gene and protein levels upon IL-1 α cytokine stimulation, to identify a mechanism which could explain our findings in the mouse models. Pancreatic fibroblasts and bone marrow-derived macrophages (BMDMs) were isolated from C57BL/6 wild-type mice and stimulated with 10 ng/mL recombinant murine IL-1 α for 24 hours. After stimulation, gene expression analysis was performed using the Affymetrix Clariom™ S Assay (mouse) on both cell types. Additionally, proteomic analysis was conducted on the fibroblasts and BMDMs using the same samples.

Transcriptome analysis revealed significant upregulation and downregulation of numerous genes in IL-1 α -stimulated fibroblasts. In contrast, only five out of 22,207 genes exhibited altered expression in IL-1 α -stimulated BMDMs (Fig. 13a). Similarly, a substantial number of proteins were differentially expressed in IL-1 α -stimulated fibroblasts, while only three proteins were downregulated in IL-1 α -stimulated BMDMs (Fig. 13b).

A closer examination of the regulated genes of interest in fibroblasts stimulated with IL-1 α provided insights into the cellular response. Notably, there was a significant increase in the expression of the gene encoding the IL-1R1 receptor, along with genes encoding pro-inflammatory cytokines and chemokines such as CCL5, CCL7, IL-6, and CXCL1, indicating a pronounced pro-inflammatory profile. Additionally, there was upregulation of genes encoding receptors for various cytokines involved in fibroblast activation and the production of extracellular matrix proteins, including IL-4, IL-13, and TGF- β . It is important to note that no profibrotic profile was detected, as evidenced by the insignificant changes in the expression of genes encoding fibrosis-associated proteins, such as collagens and fibronectins (Fig. 13c).

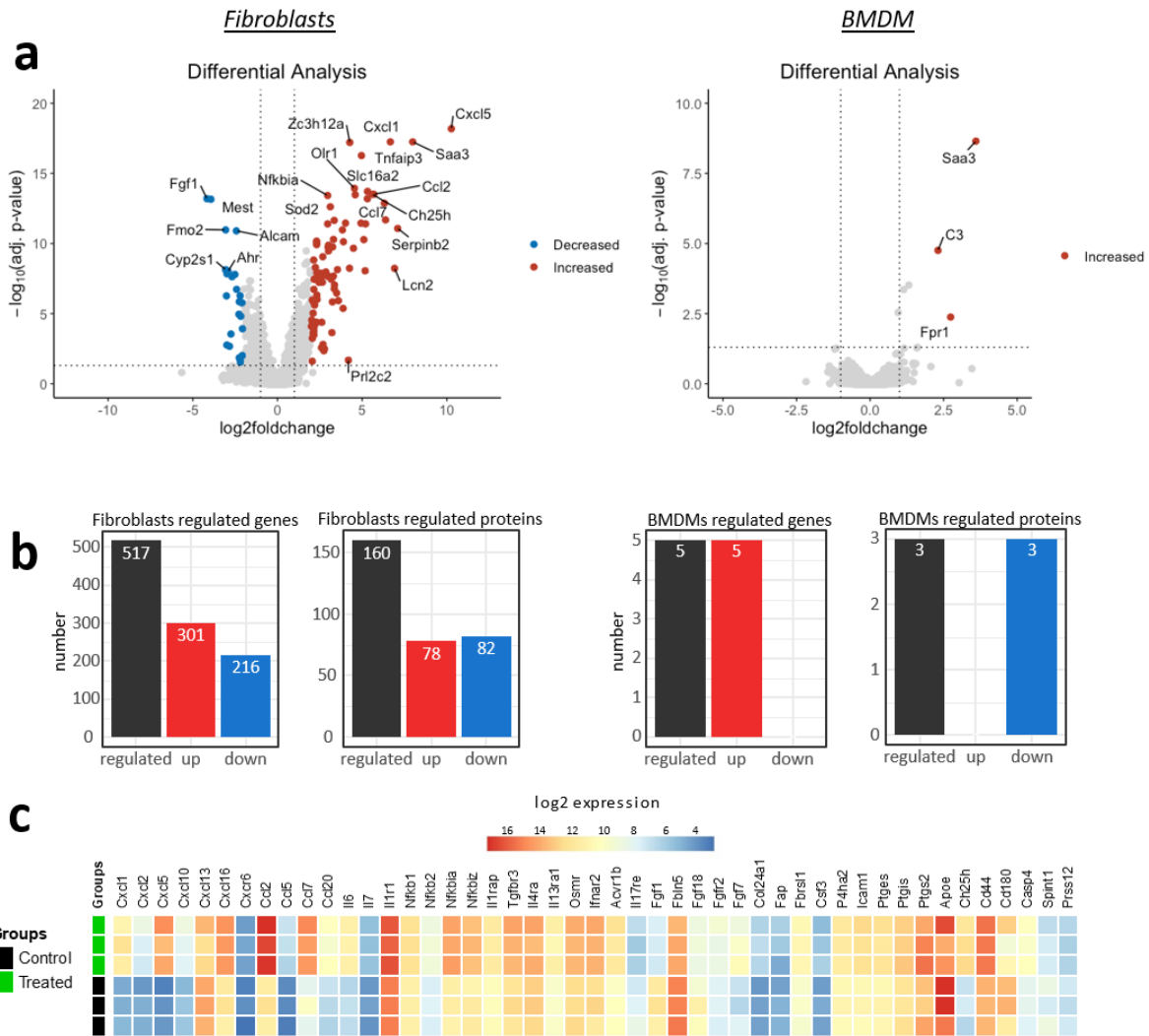


Figure 13: Transcriptome analysis of Affymetrix GeneChip array performed on cells stimulated with IL-1 α

(a) Differential gene expression analysis of isolated fibroblasts and BMDMs stimulated with IL-1 α (n=3), shown as fold change relative to untreated controls. (b) Statistical visualization of regulated genes and proteins in IL-1 α -stimulated fibroblasts and BMDMs. (c) Heatmap displaying a selection of significantly regulated genes in stimulated fibroblasts. Significantly different messenger RNA levels were defined using the following criteria: 1-way analysis of variance with Benjamini and Hochberg false discovery rate ($P \leq .05$), signal correction statistics (Ratio Builder software; $P \leq .05$), and an expression value ratio between the different conditions ≥ 1.5 -fold.

One interesting observation from the proteome analysis was the significant upregulation of the TGF β R3 receptor in fibroblasts stimulated with IL-1 α compared to unstimulated fibroblasts (Fig. 14a). The IPA pathway analysis also revealed activation of the IL-4 and IL-13 signaling pathways and a strong tendency toward Hepatic Fibrosis/Hepatic Stellate Cell Activation, which indicates a critical role of IL-1 α on pancreatic fibrosis (Fig. 14b).

Immunofluorescence labeling of NF- κ B, Ki-67, and α -SMA in isolated pancreatic fibroblasts revealed distinct localization patterns. In fibroblasts stimulated with IL-1 α , both Ki-67 and NF- κ B showed prominent nuclear localization, whereas in unstimulated cells, this nuclear presence was absent (Fig. 14c). These findings suggest that IL-1 α stimulation induces fibroblast proliferation and activates the NF- κ B signaling pathway.

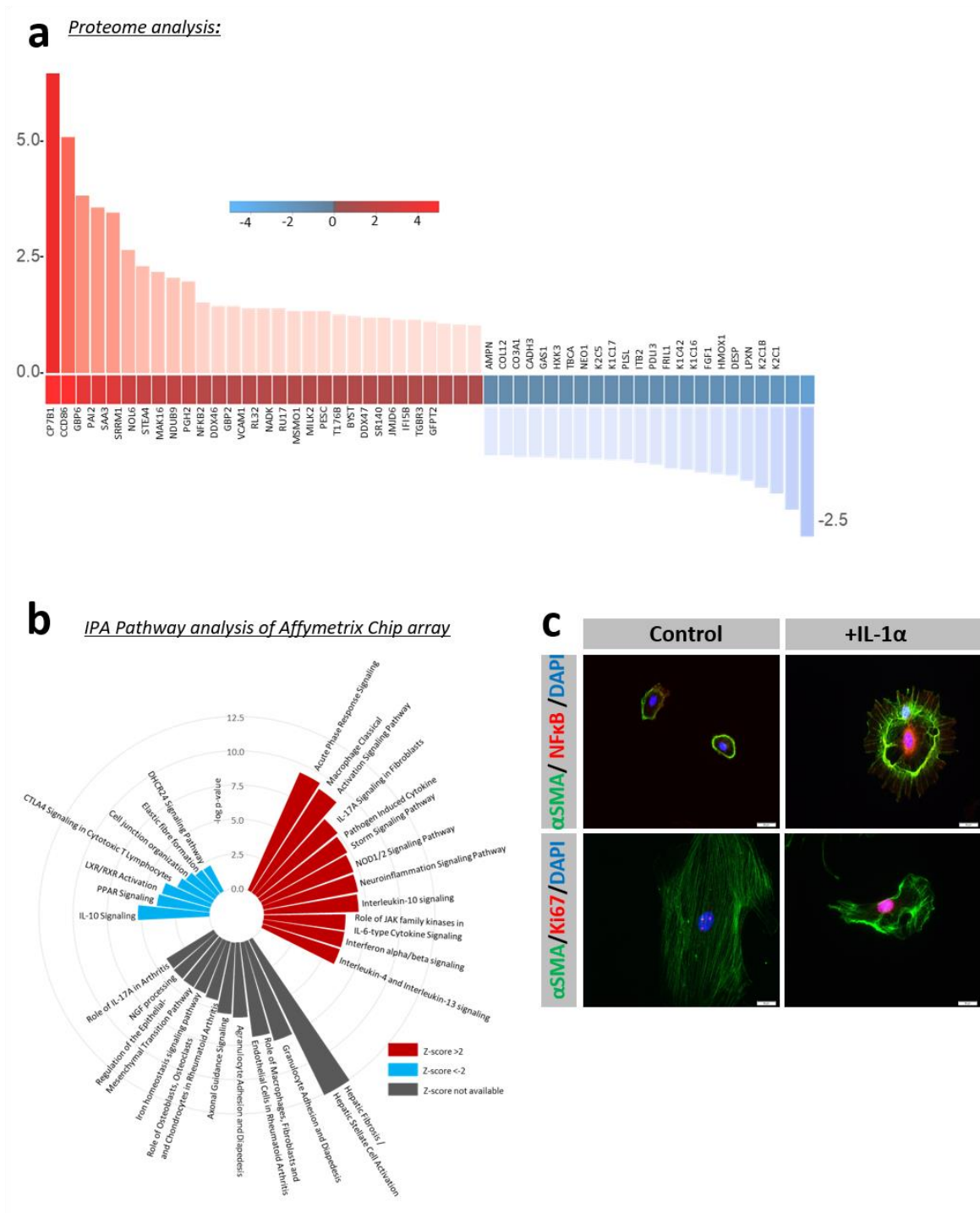


Figure 14: Proteomic and Ingenuity Pathway Analysis (IPA) of Affymetrix GeneChip array data from IL-1 α -stimulated cells, along with immunofluorescence labeling to visualize cellular responses.

(a) Proteome analysis highlighting key differentially expressed proteins in stimulated fibroblasts. (b) IPA pathway analysis of Affymetrix chip array results, illustrating signaling pathways potentially activated or deactivated in fibroblasts upon IL-1 α stimulation. (c) Immunofluorescence labeling of NF- κ B and Ki-67 in isolated fibroblasts stimulated with IL-1 α . (n=3).

Transcriptomic and proteomic profiling of fibroblasts and macrophages revealed that IL-1 α has a pronounced effect on fibroblasts, but not on macrophages. Stimulation with IL-1 α induced a pro-inflammatory phenotype in fibroblasts; however, contrary to expectations, it did not promote a clear pro-fibrotic phenotype. Instead, we observed upregulation of receptors associated with extracellular matrix protein production. These findings suggest that IL-1 α may contribute to fibrosis indirectly by priming fibroblasts rather than directly inducing a fibrotic response.

3.7 Investigating the Priming Effect of IL-1 α on Pancreatic Fibroblasts and Its Influence on Fibroblast Proliferation

To evaluate a priming effect of IL-1 α on pancreatic fibroblasts we stimulated fibroblasts under four conditions: TGF- β (10 ng/ml), IL-1 α (20 ng/ml), and co-stimulation with both TGF- β (10 ng/ml) and IL-1 α (20 ng/ml), unstimulated cells refer as controls. The expression of fibrosis markers, including *Col3a1*, *Col1a1*, *Fn1*, and *Acta2*, was assessed by qPCR. A significant increase in the expression of these genes was observed in cells co-stimulated with both cytokines, compared to cells stimulated with only IL-1 α or only TGF- β , as well as to the control (Fig. 15a).

Additionally, isolated fibroblasts were subjected to a CCK-8 proliferation assay following stimulation with 10 ng/ml IL-1 α for 24 hours. The assay revealed a significantly higher cell viability optical density in IL-1 α -stimulated cells, indicating greater cell proliferation compared to the unstimulated controls (Fig. 15b).

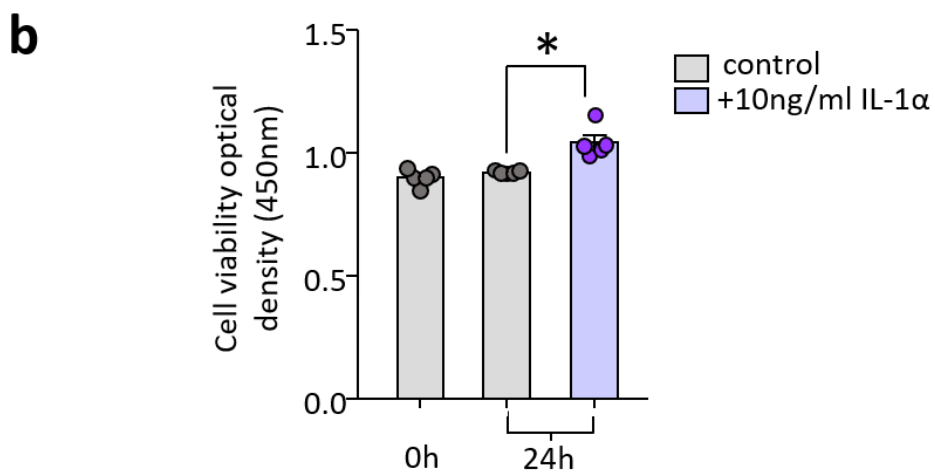
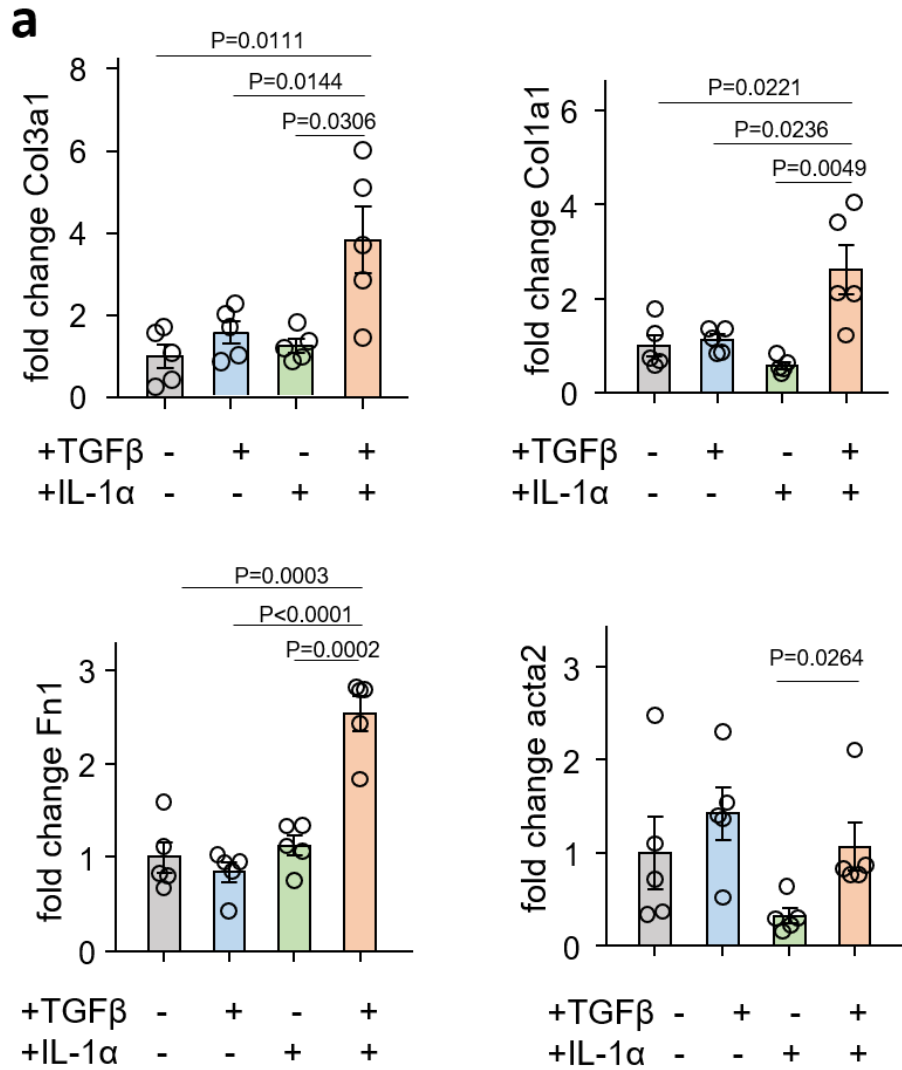


Figure 15: Assessment of priming and proliferation of fibroblasts stimulated with IL-1α

(a) qPCR analysis of fibrosis markers (n=5 for each stimulation condition) and **(b)** CCK-8 proliferation assay (n=5/group at 0h and 24h) in isolated fibroblasts stimulated with IL-1α. Statistical analysis was performed using an unpaired two-sided t-test, with significance set at *p < 0.05.

4. Discussion

Acute pancreatitis is a gastrointestinal disease marked by intrapancreatic activation of digestive proteases (4). The premature intracellular activation of trypsinogen by cathepsin B results in necrotic cell death of acinar cells (75–77). Tissue damage is accompanied by a pronounced local and systemic immune response. Current evidence suggests that the immune system, particularly innate immune cells, plays a crucial role in controlling local tissue damage and determining the severity of acute pancreatitis (5,6,48,78). Up to one third of patients with acute pancreatitis go on to develop chronic pancreatitis (20,21). During recurrent acute episodes of pancreatitis, necrotic areas in the pancreas are progressively replaced by fibrotic tissue. This process, known as the necrosis-fibrosis sequence (30), leads to chronic pain and causes irreversible, progressive damage to the pancreas (8,21,28,29,79). Recent data suggest a critical role of infiltrating immune cells, especially macrophages, in tissue remodeling (80). However, the mechanism underlying the transition from the proinflammatory to the profibrotic stage in pancreatitis remains largely unexplored. This study aimed to investigate the role of the pro-inflammatory alarmin IL-1 α in modulating pancreatitis severity and fibrosis progression using mouse models of acute and chronic pancreatitis. Building on these findings, we further investigated whether therapeutic modulation of the immune response could positively influence disease progression and serve as an option to prevent loss of organ function.

During acute pancreatitis, the immune system is activated in response to pancreatic damage. The severity of pancreatitis is determined by both the extent of tissue damage and the intensity of the immune response. In parallel with intracellular activation of trypsinogen, the immune-relevant transcription factor NF- κ B becomes activated within minutes (43,81). This activation leads to the release of pro-inflammatory cytokines and chemokines, which recruit immune cells to the site of inflammation. Destroyed acinar cells release DAMPs, which activate leukocytes via the Toll-like receptor pathway, resulting in amplification of the immune response and infiltration of leukocytes into the damaged organ (53). Inhibiting this infiltration diminishes inflammation, reduces intracellular protease activity, acinar cell necrosis, and ultimately alleviates disease progression (48).

Upon tissue injury, IL-1 α acts as a critical alarm signal, initiating the inflammatory response by recruiting innate immune cells to the site of damage, primarily through the induction of expression of pro-inflammatory cytokines and chemokines, which activate and recruit immune cells to the affected area (57,62,64). This mechanism has been shown to be central to the induction of inflammation in several diseases. In recent years, IL-1 α has emerged as a key factor in driving cutaneous inflammation (62,82–84), colon inflammation, and cancer, primarily by promoting a chronic inflammatory environment that accelerates tumor initiation and progression (62,85), cardiovascular disease (62,86), and neural inflammation (62,87). Additionally, IL-1 α plays a unique role in promoting autoinflammatory diseases, especially in a mouse model of neutrophilic dermatosis (62,84). Transgenic mice overexpressing IL-1 α in epidermal cells develop an inflammatory skin condition, underscoring the harmful effects of excessive IL-1 α production on skin inflammation (62,88). However, the role of IL-1 α in pancreatitis remains underexplored; therefore, we aimed to investigate its function more thoroughly throughout the disease progression.

A previous study from our lab showed that BMDMs co-incubated with acinar cells exhibit a pro-inflammatory profile, increasing the expression of pro-inflammatory chemokines and cytokines, including IL-1 α (5). This suggests a potential role for IL-1 α in acute pancreatitis. Our new findings confirm that IL-1 α exacerbates the severity of the disease, as demonstrated by increased pancreatic damage (Fig.7) and elevated local and systemic inflammation in its presence compared to its absence (Fig.8). IL-1 α appears to intensify acute pancreatitis through mechanisms similar to those in other diseases, where it functions as an alarmin. By inducing pro-inflammatory cytokines and activating and recruiting monocyte-derived macrophages to the site of pancreatic necrosis, IL-1 α contributes to the initiation of both local and systemic immune responses, thereby amplifying inflammation. IL-1 α plays a critical role in the activation of endothelial cells and regulates the expression of adhesion molecules such as ICAM-1 and VCAM-1, thereby facilitating the transmigration of leukocytes from the vasculature into the tissue (89,90). In our observations, the number of CCR2⁺ infiltrating cells in the pancreas is reduced, which may be attributed to diminished endothelial activation in the absence of IL-1 α .

IL-1 α is recognized as a fibroblast-activating factor, as demonstrated in studies by Suwara et al. (2013)(91). In this study, the authors show that IL-1 α , released from damaged epithelial cells, is essential for triggering inflammatory responses in human lung fibroblasts, promoting the expression of pro-inflammatory cytokines and collagen deposition, thus contributing to fibroblast activation in lung injury. These findings suggest that IL-1 α may contribute to fibrosis progression in chronic pancreatitis, prompting further investigation into its role, particularly after observing a significant increase in IL-1 α concentration in the serum of mice with chronic pancreatitis (CP) compared to controls. (Fig. 11d). We demonstrated the essential role of IL-1 α in the progression of chronic pancreatitis, as evidenced by a significant reduction in the percentage of pancreatic fibrosis and pancreatic fibroblasts (Fig. 10), as well as a decrease in the percentage of proliferating acinar cells and fibroblasts (Fig. 11b, c) in the pancreas of IL-1 α knockout mice compared to wild-type mice in the chronic pancreatitis model.

Our observations suggest that IL-1 α plays a crucial role in bridging the proinflammatory phase of pancreatitis—where it triggers immune response activation—with the profibrotic phase during disease progression, where it promotes fibrosis in the pancreas. Notably, identifying the specific cells that secrete IL-1 α in pancreatitis, as well as those expressing IL-1R1, its receptor, provides valuable insights into the underlying mechanisms of disease progression.

It is important to note that IL-1 α functions as an alarmin and is not actively released under normal conditions. Instead, it is primarily released upon cellular stress, injury, or necrosis, serving to recruit immune cells to the site of tissue damage and initiate an inflammatory response (57,64,92,93). During pancreatitis, acinar cells undergo stress due to the premature activation of their intracellular digestive enzymes, with necrosis/necroptosis being the predominant form of acinar cell death in this condition (18,94). Based on our findings, isolated acinar cells stimulated with supramaximal concentrations of the stress factor CCK release IL-1 α in a time-dependent manner, as evidenced by the significant increase in IL-1 α gene expression following stimulation (Fig.6b).

Some studies have shown that IL-1 α interacts with IL-1R1 on resident macrophages or infiltrating myeloid cells (e.g., monocytes, macrophages) at necrotic tissue sites, thereby triggering the production of proinflammatory cytokines and chemokines as part of the post-necrotic inflammatory response (92,95). However, in our investigation, we unexpectedly

found that macrophages do not respond to IL-1 α in the context of pancreatitis. We identified an entirely different site of action for this cytokine. Flow cytometry analysis of serum from mice with chronic pancreatitis revealed that monocytes showed no prominent expression of IL-1R1, whereas fibroblasts do (Fig.6d). Additionally, the colocalization of IL-1R1 and α SMA on the same cells (Fig.6c), along with the distinct localization of IL-1R1 and CD206 on different cells (Fig.6f), clearly demonstrates that fibroblasts express IL-1R1 in the pancreas of mice with chronic pancreatitis, while macrophages do not. Furthermore, our transcriptome analysis showed that bone marrow-derived macrophages (BMDM) stimulated with IL-1 α exhibited no significant changes in gene expression, whereas isolated pancreatic fibroblasts demonstrated a robust response to IL-1 α , with substantial alterations in the expression of numerous genes and proteins (Fig. 13a, b). All this evidence leads us to conclude that IL-1 α acts on fibroblasts rather than on macrophages. We fully agree with the findings of the previous studies that IL-1 α is a key player in inducing the activation and recruitment of leukocytes, mainly monocytes, to the site of damage. However, our study suggests that IL-1 α acts on cells such as fibroblasts and triggering proinflammatory signaling pathways in these cells. This process enhances the expression of proinflammatory cytokines and chemokines, which, in turn, facilitate immune cell recruitment, rather than IL-1 α directly binding to macrophages to induce inflammation (Fig. 9a).

Recent studies in pancreatic cancer highlight the critical role of fibroblasts in tumor development. Similar to chronic pancreatitis, pancreatic carcinoma is characterized by an immunosuppressive fibroinflammatory microenvironment (28,96,97). Cancer-associated fibroblasts (CAFs) contribute significantly to carcinogenesis and tissue remodeling by promoting fibrosis (98). One of the major challenges in treating pancreatic cancer is its resistance to chemotherapy. Research from David Tuveson's group has demonstrated that this resistance is primarily due to the dense fibrotic stroma surrounding the tumor, which limits vascularization and hinders drug delivery. The chemoresistance, therefore, does not originate from intrinsic tumor cell properties, but rather from the physical barrier created by the fibrotic tissue, which prevents therapeutic agents from reaching the cancer cells (99). Recent data indicate that elevated IL1A expression in tumor tissue correlates with poor prognosis and disease progression in pancreatic ductal adenocarcinoma (100). This association may be attributed to increased fibrosis driven by IL-1 α . Similarly, in colorectal cancer, IL-1 α has been shown to directly influence CAF activation, tumor progression, and

stromal development (101). These findings are consistent with our own data and support a pro-fibrotic role of IL-1 α in chronic pancreatitis as well.

To further elucidate the mechanisms underlying IL-1 α -mediated fibrosis enhancement, we investigated how IL-1 α interacts with its receptor, IL-1R1, on fibroblasts. Initially, we hypothesized that IL-1 α directly increases the expression of procollagen synthesis. However, our transcriptomic analysis clearly demonstrated that stimulation of isolated pancreatic fibroblasts with IL-1 α did not alter the expression of fibrosis markers (Fig. 13c). This suggests that IL-1 α promotes fibrosis through an alternative mechanism.

A particularly notable observation from our transcriptomic data was the significant upregulation of receptors for specific cytokines and growth factors, including TGF β R, IL-4R, and IL-13R (Fig. 13c). This finding led us to propose that IL-1 α primes fibroblasts, enhancing their responsiveness to subsequent signals from other profibrotic cytokines. This hypothesis was confirmed by the significant increase in the expression of fibrosis markers, such as *Col1a1*, *Col3a1*, and fibronectin, in fibroblasts co-stimulated with IL-1 α and TGF β (Fig. 15a). These results provide strong evidence that IL-1 α enhances fibrosis through an indirect priming mechanism rather than direct induction of collagen synthesis.

Previous studies have demonstrated that interleukin-1 alpha (IL-1 α) plays a significant role in promoting the proliferation of epithelial cells (102,103) and fibroblasts (104). In our study, we observed a marked decrease in the percentage of pancreatic fibroblasts in the absence of IL-1 α in the context of chronic pancreatitis. This finding led us to investigate whether IL-1 α also contributes to the proliferation of fibroblasts in the pancreas. To address this, a CCK-8 proliferation assay was conducted on isolated pancreatic fibroblasts stimulated with IL-1 α . The results clearly indicated that IL-1 α induces fibroblast proliferation, thereby enhancing the growth of the fibroblast population (Fig. 15b). These findings suggest that IL-1 α plays a crucial role in driving fibrosis in chronic pancreatitis by activating and priming fibroblasts and promoting their proliferation. The following figure provides a comprehensive summary of IL-1 α secretion and its role in pancreatitis, based on our recent observations (Fig. 16).

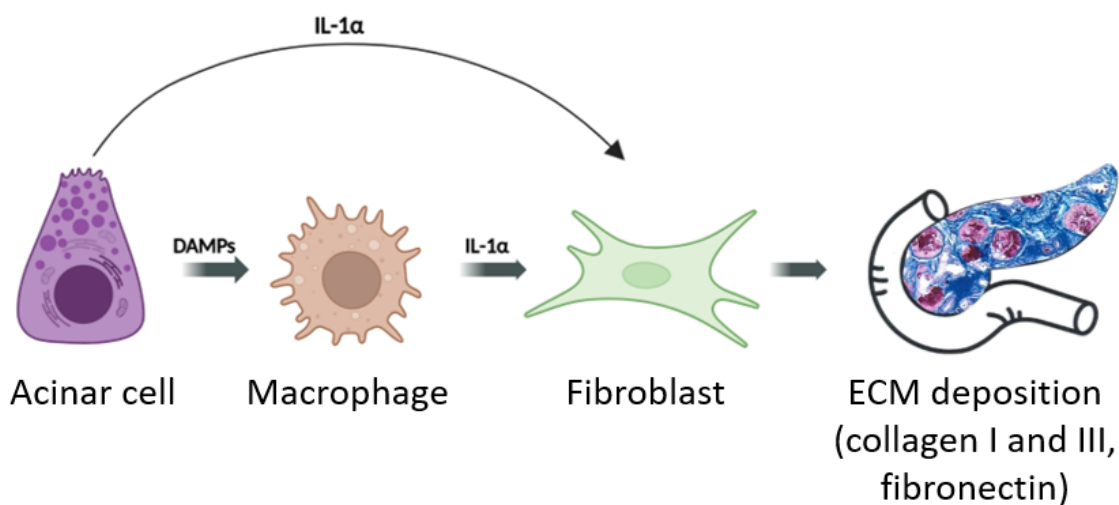


Figure 16: Secretion of IL-1 α by damaged acinar cells and activated macrophages, along with its effects on fibroblasts and the promotion of pancreatic fibrosis.

The significance of our findings lies in the fact that, contrary to previous beliefs (29,105,106), fibrosis in the pancreas does not emerge solely in the later stages of the disease. Instead, it begins at early time points when pancreatic cells are still in the proinflammatory phase, releasing signaling molecules and alarmins, such as IL-1 α , to regulate and mitigate necrosis progression.

The proinflammatory cytokine IL-1 α , released within hours of disease onset, interacts with pancreatic fibroblasts and promotes fibrosis through the mechanisms described above. Furthermore, the activation and proliferation of fibroblasts do not require the involvement of immune cells such as lymphocytes, macrophages, neutrophils, or platelets. Our findings demonstrate that injured or necrotic acinar cells themselves directly release the proinflammatory cytokine IL-1 α , thereby activating the fibrotic pathway. Additionally, the release of IL-1 α from both acinar cells and classically activated macrophages enhances its effects on pancreatic fibroblasts, further accelerating pancreatic fibrosis (Fig. 6).

This links acinar cell death during acute episodes of pancreatitis directly to the induction of wound healing and fibrosis, providing an explanation for the necrosis-fibrosis sequence. Therefore, fibrosis is a direct consequence of acinar cell necrosis in the pancreas. Since IL-1 α is predominantly released by necrotic cell death rather than apoptotic cell death, acinar cell

apoptosis does not typically lead to significant fibrogenesis. Criscimanna et al. used a mouse model expressing the diphtheria toxin receptor specifically on pancreatic acinar cells. Upon administration of diphtheria toxin, approximately 95% of the acinar cells underwent apoptosis. Three weeks later, they observed regeneration of the exocrine tissue without signs of fibrosis (107). This finding further suggests that the onset of fibrosis is triggered by acinar cell necrosis, whereas acinar cell apoptosis appears not only immunologically silent but also anti-fibrotic in nature. The form of acinar cell death seems to influence not only the progression of acute pancreatitis but also the development of chronic disease (94,108).

The anti-inflammatory agent anakinra functions by binding to the IL-1R1 receptor, thereby preventing the interaction of IL-1 α and IL-1 β with this receptor. Anakinra is approved for its efficacy in treating inflammatory diseases, including rheumatoid arthritis (RA) and cryopyrin-associated periodic syndrome (CAPS)(57). A notable study demonstrated that following anakinra administration (IL-1 receptor antagonist), inflammation significantly decreased in the lungs of both murine models and human cystic fibrosis patients. The study reported a reduction in lung inflammation, neutrophilic infiltration, and microbial burden, suggesting that targeting IL-1 signaling could help control excessive lung inflammation in cystic fibrosis and may have a protective effect against fibrosis (109). Furthermore, another study investigated anakinra's effects in diabetic rats. Histological analyses revealed that anakinra treatment significantly reduced cardiac fibrosis compared to untreated diabetic controls (110). An additional study evaluated the therapeutic potential of Anakinra in a rat model of acute pancreatitis and indicated that Anakinra significantly reduced pancreatic tissue injury and apoptosis (111). Interestingly, our study also demonstrated a protective role of anakinra in both acute and chronic pancreatitis. We observed a significant reduction in the severity of acute pancreatitis in mice treated with anakinra compared to controls (Fig.9). Additionally, the percentage of pancreatic fibrosis and fibroblasts was notably lower in anakinra-treated mice compared to untreated mice with chronic pancreatitis (Fig.12). These findings suggest that anakinra could be a promising therapeutic option for reducing pancreatitis severity and preventing the progression of pancreatic fibrosis.

IL-1 α cytokine, released within a few hours of pancreatitis onset by damaged acinar cells and macrophages activated by DAMPs, is no longer able to bind to its receptor IL-1R1 on

the surface of pancreatic fibroblasts. As a result, IL-1 α -induced fibrosis is blocked, leading to a reduction in the production of extracellular matrix components such as collagen I, collagen III, and fibronectin. This ultimately contributes to a decrease in disease severity (Fig. 17).

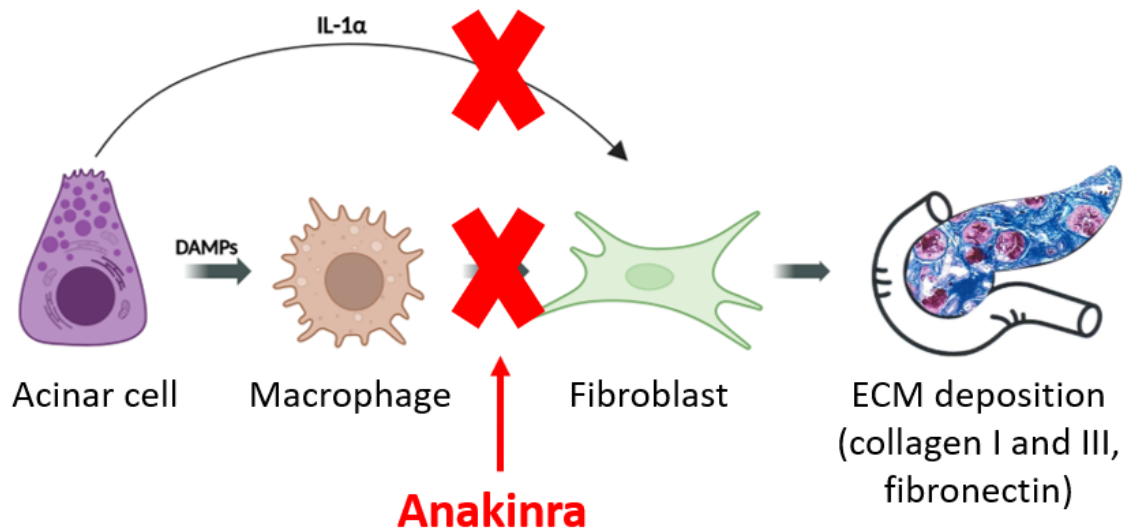


Figure 17: Inhibition of IL-1 α binding to fibroblasts and prevention of IL-1 α -dependent pancreatic fibrosis progression.

One advantage of Anakinra over monoclonal antibodies like Canakinumab, which targets IL-1 β , is its relatively short half-life of just a few hours, while Canakinumab maintains its blocking effect for several days. In acute pancreatitis, the disease course can shift to a severe form, often triggering an anti-inflammatory counter regulation (CARS)—a compensatory anti-inflammatory response syndrome (112). This immunosuppression could be exacerbated if long-acting anti-inflammatory agents, such as Canakinumab, remain active in circulation for extended periods.

Previous research has shown that inhibiting IL-1 β , particularly through the use of caspase-1 inhibitors, can significantly reduce mortality rates in rat models of severe acute pancreatitis. This approach has been found to alleviate pancreatic necrosis and injury, while also decreasing systemic inflammation demonstrated by less neutrophil infiltration in both pancreatic and lung tissues (113). However, we propose that in chronic pancreatitis (CP), IL-1 β plays only a minor role in disease severity compared to IL-1 α . Whereas during severe forms of pancreatitis the release of IL-1 β affects disease outcome (5,6,53,114), the role of IL-1 β in chronic form of pancreatitis is not well investigated. This is primarily due to the significantly

higher secretion and abundance of IL-1 α in CP-affected mice. Cytokine measurements in serum revealed that IL-1 α concentrations were markedly elevated in CP mice compared to controls, whereas IL-1 β levels were nearly undetectable (Fig. 11d). One possible explanation is that IL-1 α is directly released by necrotic acinar cells and macrophages, whereas IL-1 β is nearly exclusively released by macrophages. Moreover, the release of IL-1 β requires two signals that lead to pyroptotic cell death, which is more closely associated with severe inflammatory responses. Given this low abundance, it is unlikely that IL-1 β exerts a significant functional impact in chronic pancreatitis.

Furthermore, a previous finding from our group supports this hypothesis, demonstrating that the incubation of bone marrow-derived macrophages (BMDMs) with damage-associated molecular patterns (DAMPs) resulted in a substantially greater increase in IL-1 α gene transcription compared to IL-1 β , with the upregulation of IL-1 α being approximately twice as high as that of IL-1 β (5).

Therefore, while the reduction in disease severity observed with anakinra treatment can be attributed to the inhibition of both IL-1 α and IL-1 β binding to IL-1R1, our findings suggest that IL-1 α is the primary mediator of inflammation and fibroblast activation in chronic pancreatitis.

5. Limitations

A limitation of our study is that, although we observe an effect on pancreatic fibrosis in IL-1 α -deficient or Anakinra-treated animals, we only note a reduction in fibrosis rather than its complete absence. This is likely due to the involvement of multiple signaling pathways in tissue remodeling, such as Th2 cytokines IL-4 and IL-13, and growth factors like TGF β or FGF2, which play key roles in the production of extracellular matrix proteins. Another limitation is that we did not examine organ regeneration in detail. While we demonstrate a reduction in pancreatic fibrosis, this does not necessarily imply that organ regeneration is enhanced as a result.

Although the results of preclinical experiments are promising, further clinical studies are necessary.

For acute pancreatitis treatment, the primary goal is to reduce systemic inflammation to prevent a severe form of the disease. For the chronic form of the disease the aim is to reduce the development of pancreatic fibrosis and restore organ function. Anakinra seems to be a promising treatment option to tackle both by targeting pancreatic fibroblasts. This drug blocks the IL-1R1 receptor on the surface of these cells, preventing the binding of IL-1 α and IL-1 β . Since the pancreas is rich in proteases including trypsin, chymotrypsin, elastase and carboxypeptidase, there is a possibility that these enzymes may degrade the anakinra molecule before it reaches its target and exerts its intended effect. To address this concern, further experiments are needed to evaluate the drug's stability in the human body.

6. Summary

Pancreatitis is a common gastrointestinal disorder, till today its underlying pathomechanisms are not fully understood. The immune system, particularly the innate immune response, plays a pivotal role in the progression of the disease. The initial local immune response has been well characterized: damaged acinar cells release damage-associated molecular patterns (DAMPs), which in turn trigger the recruitment and activation of innate immune cells, especially macrophages and neutrophils, into the pancreatic tissue.

Once recruited and activated, immune cells, mainly macrophages, release pro-inflammatory cytokines and chemokines, including the alarmin IL-1 α , to signal tissue damage in the pancreas and amplify the immune response. This study demonstrated that IL-1 α is released not only by activated macrophages, but also by necrotic acinar cells during pancreatitis. IL-1 α plays a central role in the innate immune signaling and inflammation by activating endothelial cells and fibroblasts. In this study we could show that IL-1 α promote the proliferation and priming of pancreatic fibroblasts, thereby contributing to the development of pancreatic fibrosis in the context of chronic pancreatitis.

In both acute and chronic pancreatitis models, disease severity was significantly reduced in the absence of IL-1 α . This was evident from decreased serum amylase and lipase activity, reduced edema, diminished immune cell infiltration, and less acinar cell necrosis in acute

pancreatitis. In the chronic model, this was reflected by a lower degree of fibrosis and fewer pancreatic fibroblasts.

Moreover, treatment of mice with Anakinra, an anti-inflammatory IL-1 receptor antagonist, produced effects similar to those observed in IL-1 α knockout experiments. This included reduced pancreatic tissue damage—such as necrosis and inflammation—in acute pancreatitis, and attenuated fibrosis progression in chronic pancreatitis.

These findings suggest that blocking the IL-1R1 signaling pathway has a positive influence on disease progression and may represent a promising preventive therapeutic approach. Specifically, it could help limit the production of extracellular matrix components (e.g., Col1a1, Col3a1, and fibronectin 1) and thereby reduce the severity of pancreatitis.

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Eigenständigkeitserklärung

Eigenständigkeitserklärung

(Diese Erklärung ist zu unterschreiben und in die Dissertationen einzubinden.)

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

Unterschrift des*der Promovend*in

A handwritten signature in blue ink that reads "Hata". The letters are cursive and connected.

Curriculum Vitae

List of Publications

Glaubitz J., Wilden A., Frost F., Ameling S., Homuth G., **Mazloun H.**, Rühlemann M.C., Bang C., **Aghdassi A.A.**, Budde C., Pickartz T., Franke A., **Bröker B.M.**, **Voelker U.**, Mayerle J., Lerch M.M., Weiss F.U., **Sendler M.** Activated regulatory T-cells promote duodenal bacterial translocation into necrotic areas in severe acute pancreatitis. 2023 Jul;72(7):1355-1369. doi: 10.1136/gutjnl-2022-327448. Epub 2023 Jan 11.

Glaubitz J., **Asgarbeik S.**, Lange R., **Mazloun H.**, Elsheikh H., Weiss F.U., **Sendler M.** Immune response mechanisms in acute and chronic pancreatitis: strategies for therapeutic intervention. 2023 Oct 10:14:1279539. doi: 10.3389/fimmu.2023.1279539.eCollection 2023.

Conference contributions

❖ 12th Autumn School 'Current concepts in Immunology', Merseburg, 10.-15.10.2022
"Proteolytic Processing of Cytokines of IL-1 Family Regulating the Immune Response during Experimental Pancreatitis". (Poster)

❖ German Pancreatic Club (DPC), LMU Munich, Germany, 02.-04.03.2023
"IL1 α acts as alarmin in acute pancreatitis and regulates inflammatory response". (Poster)

❖ German Pancreatic Club (DPC), Bonn, Germany, 15.-17.02.2024
"IL-1 α activates pancreatic Fibroblasts and regulates Tissue Fibrosis during Chronic Pancreatitis" (E-Poster)

❖ European Pancreatic Club (EPC), Santiago, Spain, 26.-29.06.2024
"IL-1 α promotes fibroblast activation and regulates tissue fibrosis during chronic pancreatitis". (Poster)

❖ Proteases in inflammation and infection, Warnemünde, Germany, 25.-27.08.2024
"IL-1 α promotes fibroblast activation and regulates tissue fibrosis during chronic pancreatitis". (Poster + Oral Presentation)

❖ German Pancreatic Club (DPC), Heidelberg, Germany, 27.02-01.03.2025
"IL-1 α promotes fibroblast activation and regulates tissue fibrosis during chronic pancreatitis". (Oral Presentation)

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