The Pathogenesis of Chronic Pancreatitis

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1 Pain Mechanisms in Chronic Pancreatitis

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Abstract Chronic abdominal pain remains a major clinical challenge in chronic pancreatitis (CP) and is present in up to 90% of the patients. It is associated with a poor life quality, an increased health resource utilization and is the primary cause of hospitalization Lieb et al. (Aliment Pharmacol Ther 29:706–19, 2009). The etiologies of pain in CP are increasingly better understood and likely involve multiple mechanisms. The focus of this chapter is to provide an overview of the mechanisms involved in chronic pancreatic pain. First, the traditional view of pain in CP is discussed where pain is thought to arise due to mechanical problems such as obstruction of the pancreatic gland. Although this theory is widely accepted and forms the theoretical background for invasive treatments of pain it is largely undocumented and has been challenged by recent research where no uniform associations between morphological changes and pain exist. The next section provides an overview of a novel neurobiological understanding of pain in CP, which is shifting the focus of pain from mechanical problems towards changes in peripheral and central pain processing. This has substantial consequences for treatment and may result in a paradigm shift of pain management of CP in a foreseeable future. Finally, we briefly discuss extra-pancreatic causes of pain in associated with CP, which are important to diagnose, as they are often easy to treat.

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1.1 "Plumbing" Problems

Traditionally, it is generally accepted that pain is generated by increased pressure in the pancreatic ductal system or in the pancreatic parenchyma due to duct obstruction, stricture and/or peripancreatic fibrosis (Lieb et al. 2009; Anderson et al. 2015). Thus the "the plumbing theory" has been the theoretical background for most interventions with the common purpose to alleviate

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the increased pressure through different surgical and endoscopic drainage procedures (Anaparthy and Pasricha 2008).

1.1.1 Ductal Pressure and Pain

White and co-workers described the first case report of a relationship between pancreatic duct pressure and pain in 1970 (White and Bourde 1970). Following acute necrotizing pancreatitis, a patient underwent open necrosectomy and with a drainage catheter communicating the pancreatic duct, he reproducibly reported pain with increased ductal pressure (White and Bourde 1970). Later studies attempted to verify the consumptioccccn while most of them were flawed by inappropriate methodology (Fasanella et al. 2007). In a study by Sato and co-workers reported that compared to patients with gastric cancer, intraoperative measured pancreatic ductal pressure significantly increased in CP patients (Sato et al. 1986). Moreover, another study reconfirmed the ductal hypertension during endoscopic management (Okazaki et al. 1986). However other studies with the same manometry reported no ductal hypertension (Laugier 1994; Rolny et al. 1986; Ugljesić et al. 1996; Vestergaard et al. 1994), and demonstrated no difference in pressure levels referring to pain (Novis et al. 1985), which was supported by another study with the finding that ductal pressure did not precisely predict the ductal decompression (Renou et al. 2000). Furthermore, the link between ductal hypertension and pain in CP remains speculative as the mechanism was unclear.

1.1.2 Parenchymal Pressure and Pain

Increased parenchymal pressure measurement of the pancreas has also been suggested as a cause of pancreatic pain. A pioneer study was done by Ebbehøj and co-workers, which depicted a novel needle probe inserted directly into the pancreatic parenchyma to measure parenchymal pressure (Ebbehøj et al. 1984). In a cohort of 39 CP patients, patients with pain resulted in higher intrapancreatic pressure and pain was relieved after surgical drainage (Ebbehøj et al. 1990a). In a 1-year study, it was reported that recurrent pain can cause rebound of increased intrapancreatic pressure (Ebbehøj et al. 1990b). However, these findings were flawed by inclusion of patients without pain and not reproduced in a more recent study using a similar technique (Manes et al. 1994).

The pathophysiological link between increased intrapancreatic pressure and pain has been described as a "compartment-like syndrome" (Fasanella et al. 2007). In an animal model of CP, increased interstitial pressures, diminished blood flow and ensuing tissue acidosis were documented after stimulation with cholecystokinin and secretin (Patel et al. 1995). A human controlled experiment reported in the same paper drew a conclusion that the CP patients demonstrated significantly more acidotic tissue, which was suggested to be the result of secondary ischemia mimicking the pathophysiology underlying muscular compartment syndrome (Zhu et al. 2011; Schwartz et al. 2013) (described further below). Nonetheless, it must be underlined that these findings have never been reproduced.

1.1.3 Pancreatic Morphology and Pain

As measurement of pancreatic pressure can be invasive and potentially harmful, most decisions regarding surgery or endotherapy to relieve pain in the clinic rely on morphological abnormalities of the pancreas, such as pancreatic duct stricture, obstruction or pseudocysts (Lieb et al. 2009; Fasanella et al. 2007; Warshaw et al. 1998). Yet the correlation between morphological changes and pain have been challenged by a number of studies demonstrating no obvious morphological difference referring to pain, and even severe pain (Bornman et al. 1980; Lankisch et al. 1993; Malfertheiner et al. 1987; Jensen et al. 1984). The largest study examining the association between abdominal imaging features and pancreatic pain was done by Wilcox and co-workers (2015). Of the 518 CP patients examined, 427 reported abdominal pain from CP during the year before enrolment and the pattern (i.e. constant vs. intermittent pain) and severity of pain were independent of morphological changes.

Diffusion weighted imaging with MR imaging, which can provide information of atrophy, ductal pathology and microstructure of the tissue, was applied in a study by Frøkjær et al. In this study, the association between pathological imaging and pain came to the same result in agreement with the aforementioned study but morphological changes were associated with pancreatic function (Balci et al. 2009; Frokjaer et al. 2013).

Taken together, pancreatic morphology is not associated with pain in CP and, as such, the rationale for invasive treatment solely based on the results of imaging is questionable.

1.2 "Wiring" Problems

In patients with CP, inflammation and progressive replacement of the normal pancreatic tissue with fibrosis can lead to changes in the function and morphology of intrapancreatic nerves. Collectively these processes have been referred to as "wiring problems" (Moran et al. 2015) and represents a wide spectrum of changes in peripheral nociception and central pain processing which is discussed in detail below.

1.2.1 Peripheral Changes

To understand the neurobiological perspective on pain in CP, a basic knowledge about pain perception and processing is required Depending on the excitability of the neural membrane, the stimulus sensed by a variety of nociceptors may lead to generation of an action potential, which travels along afferent nerves to the spinal end of the nerves in the dorsal horn to trigger the release of neurotransmitters, which cross the synapse and activate secondary neurons that transmit the noxious stimulus to the brain through different pathways, ultimately resulting in the sensation of pain (Anaparthy and Pasricha 2008).

Peripheral Sensitization

Nerve growth factor, normally expressed by islets in the pancreas, is amongst the most important and well-characterized neuropeptides involved in growth, regulation and proliferation of certain neurons (Woolf et al. 1994). In rats with CP, it is upregulated. Nerve growth factor can not only significantly increase nociceptor excitability, pancreatic hyperalgesia and referred pain to somatic structures but also upregulate the nociceptor transient receptor potential vanilloid-1 in animal model of CP, as well as in humans with CP (Xu et al. 2007; Toma et al. 2000; Hartel et al. 2006). Thus, in preliminary studies, antagonists for transient receptor potential vanilloid-1 have been developed and proved to be effective in humans with neuropathic pain. However, hyperthermia, is still a concern of transient receptor potential vanilloid-1 antagonism (Wong and Gavva 2009).

Release of cytokines and chemokines, such as IL-8 and fractalkine, from immune cells infiltrating the pancreas during CP has been associated with pancreatic pain (Ceyhan et al. 2009a; Di Sebastiano et al. 1997). Specifically, compared to patients with painless CP, the number of mast cells can reach a 3.5 fold increase in those with pain (Hoogerwerf et al. 2005; Esposito et al. 2001). A proposed mechanism is increased activation of protease-activated receptor 2, triggered by tryptase released from the mast cells (Hoogerwerf et al. 2005). Other upregulated, pro-inflammatory cytokines have also been suspected to play a role in the pain generation in CP, and in some cases this upregulation and resulting pancreatic neuritis may increase pain intensity and/or frequency (Bockman et al. 1988; Keith et al. 1985; Ceyhan et al. 2009b) (for a thorough review see (Fasanella et al. 2007)).

Additionally, upregulation of neurotransmitters involved in pain signalling at the central end of the nociceptor, such as calcitonin gene-related peptide, substance P and brain-derived neurotrophic factor, has been demonstrated in animals with CP along with increased sensory nerve excitability, and pharmacological blockade of these receptors has likewise been shown to reduce pain (Hughes et al. 2011; Liu et al. 2011; Büchler et al. 1992).

These functional alterations render the nociceptors more sensitive to further stimulation (Gebhart 2000; Anand et al. 2007). This so called *peripheral sensitization*, results in an increased

barrage of pain signals to the spinal cord (Woolf and Salter 2000), which is believed to increase clinical pain intensity, can be an important factor in the pathogenesis of pain in CP (Bockman et al. 1988; Keith et al. 1985; Ceyhan et al. 2009c).

Peripheral Neuropathy

Besides the changes on the molecular level, CP is also associated with prominent morphological and/or functional alterations of pancreatic nerves (Bockman et al. 1988; Ceyhan et al. 2009c). These changes are collectively referred to as "neural plasticity" at the cellular (neuronal) level. The characteristic features of pancreatic nerves in human CP are increased neural density (neural sprouting), increased neural size (neural hypertrophy), and perineural inflammations (neuritis) (Ceyhan et al. 2009c; Friess et al. 2002; Demir et al. 2015). In addition to the morphological alterations it has been demonstrated that nerves in patients with CP contain fewer sympathetic or adrenergic nerve fibres than normal pancreatic tissue—a phenomenon referred to as neural remodelling (Ceyhan et al. 2009b). Although these changes in many cases have been shown to relate to sensation in CP, the mechanisms and interactions with the functional neural changes are not fully understood (Demir et al. 2015) (Table 5.1).

Table 5.1 Peripheral pain mechanisms in chronic pancreatitis and its associated experimental evidence

Mechanism	Experimental evidence
Enhanced nociception	Upregulated transient receptor potential vanilloid-1
	Increased activation of protease-activated receptor 2
Upregulated neurotransmitter expression	Increased nerve growth factor and expression of calcitonin gene-related peptide, substance P and brain-derived neurotrophic factor
Pancreatic neuritis	Increased number of immune cells e.g. pancreatic mast cells, increased IL-8 and fractalkine level
Pancreatic neuropathy	Neural sprouting, neural hypertrophy

1.2.2 Central Changes

Central Sensitization

An augmented signalling of noxious stimuli to the spinal cord induces increased responsiveness of central pain transmitting neurons and thereby increases the gain in the whole pain system. This phenomenon is known as *central sensitization* leading to intense peripheral noxious stimuli, tissue injury, or nerve damage (Woolf 2011; Latremoliere and Woolf 2009). The process it typically characterised by increased excitability, expansion of the dorsal horn neurons receptive field and by sprouting of non-nociceptive afferents into "pain-specific" areas of the spinal cord. These functional and structural changes explain the clinical and experimental findings associated with central sensitization:

- primary hyperalgesia: increased sensitiveness to painful stimuli of the diseased organ (e.g. increased sensitiveness to stimulation of the pancreas)
- secondary hyperalgesia: a receptive field expansion that enables input from non-injured tissue to produce pain (e.g. increased sensitiveness to stimulation of visceral organs remote to the pancreas such as the rectosigmoid or somatic structures)
- allodynia: pain in response to a non-noxious stimulus (e.g. postprandial pain reported by patients with CP)

Several experimental human pain studies have reported increased areas of referred pain and augmented pain sensitiveness was seen in CP patients corresponding to primary and secondary hyperalgesia as discussed above (Dimcevski et al. 2007). Along this line, additional studies reported decreased pain thresholds to somatic stimulation of muscle and bone as well as stimulation of the rectosigmoid (Olesen et al. 2010a; Buscher et al. 2006). The latter reflects a special form of secondary hyperalgesia (viscero-visceral hyperalgesia) seen in visceral pain disorders accompanied by central sensitization.

Many patients with CP report postprandial pain, which, in addition to changes in ductal or

parenchymal pressure mediated by humeral mechanisms, may also reflect allodynia triggered by non-noxious mechanical stimuli when the food passes the upper segments of the gastrointestinal tract in close proximity to the pancreas. In a sensitized pain-system, food passage may activate previous non-nociceptive neurons that now covey noxious information due to e.g., sprouting into pain signalling areas of the spinal cord. This again leads to allodynia perceived as postprandial pain by the patient.

One of the best characterised mechanisms involved in central sensitization is activation of the N-methyl-D-aspartic acid (NMDA) receptor, thus revealing a key involvement of glutamate in this process (Willert et al. 2004). Blocking of the NMDA receptor by ketamine has been shown to reverse hyperalgesia associated with CP in an experimental study (Bouwense et al. 2011a) and ketamine is currently under investigation in a randomised placebo controlled-trial of painful CP (Juel et al. 2015). Also, changes in ion channel properties have been shown to play a key role in the process of central sensitization. These can be modulated by gabapentoids, such as gabapentin and pregabalin, which target the pre-synaptic voltage-dependent calcium channels. In patients with CP, pregabalin is effective as an adjuvant treatment of pain in patients with CP and reverse associated primary and secondary hyperalgesia. Interestingly its effect can be predicted by segmental hyperalgesia of the pancreatic viscerotome (the upper abdominal skin area sharing spinal innervation with the pancreatic gland) and, as such, pregabalin treatment can be tailored to the individual patients pain profile (Olesen et al. 2013a).

Taken together, these clinical, experimental, and pharmacological findings characterise a generalised hyperalgesic state of the pain system in patients with CP and likely mirrors widespread sensitization of central pain pathways. The abnormalities seem to be linked to disease severity and at some point may become independent of the peripheral nociceptive input (see discussion later) (Bouwense et al. 2013).

Abnormal Patterns of Cortical Activity

Several studies have indicated that deafferentation, chronic pain, and hyperalgesia, as seen in CP patients, are associated with a functional reorganisation of the brain areas involved in sensory processing (Flor et al. 2006). Accordingly, people with arm or hand amputations show a shift of the mouth into the hand representation in the primary somatosensory cortex, with the quantity of cortical reorganisation being correlated with subjective pain ratings (Flor et al. 1995). In patients with CP, pancreatic nerve damage and modulation may to some degree mimic the peripheral nerve pathology seen in patients following amputations. Along this line, experimental pain studies have indicated that chronic pain and hyperalgesia is associated with functional reorganisation of the visceral sensory cortex (Dimcevski et al. 2007; Olesen et al. 2010b; Lelic et al. 2014). Hence, CP patients show reorganisation of the brain areas involved in visceral sensory processing. In addition, the evidence of impaired habituation to noxious stimuli in CP patients possibly reflecting a cortical neuronal hyperexcitability (Olesen et al. 2013b). Functional reorganization and hyperexcitability may be reversed by transcranial magnetic stimulation and a sham-controlled randomised trial has documented the effectiveness of this technique for pain alleviation in patients with CP.

The thalamus, as a critical relay site in the sensory system, has been implicated in chronic pain. Hence, a disturbance of the thalamocortical interplay evidenced by global changes in the rhythmicity of the cerebral cortex was observed in patients with neuropathic pain of mixed origin (Sarnthein et al. 2006). Parallel findings were observed in CP patients in studies based on spectral analysis of visceral evoked brain potentials and resting state electroencephalography (Olesen et al. 2011; Drewes 2008). Remarkably, changes in brain oscillations following pregabalin treatment have been associated with its analgesic efficacy (Graversen et al. 2012). Thus, in addition to a spinal effect on central sensitization, the analgesic effect of pregabalin may also be mediated by supraspinal mechanisms.

Structural Cortical Alterations

With advanced imaging technology, the correlation of structural cortical alterations and hyperexicitability was found. In one study, diffusion

weighted MRI demonstrate the link between microstructural changes in the insular and frontal brain areas and clinical pain intensity and functional scores (Frøkjær et al. 2011). Pain intensity was proportional to the severity of microstructural abnormalities (Mullady et al. 2011). In another MRI based cortical volumetry study, a reduced brain areas involved in visceral pain processing suggested a central neurodegenerative response to severe and chronic pain (Frøkjær et al. 2012). Whether such structural changes represent specific signatures of pancreatic pain has yet to be determined, but evidence from other chronic pain diseases suggest that morphological changes of brain structure may be unique for different pain conditions and, as such, it suggests the possibility of unique therapies by targeting the underlying specific pathways for each type of chronic pain (Apkarian et al. 2011).

Changes in Spinal Interneurons and Pain Modulation

The pain system has several inherent mechanisms whereby inflowing pain signals are modulated. Among many mechanisms, inhibitory spinal interneurons and descending modulatory pathways from the brain stem and higher cortical structures plays a key role. Such endogenous pain modulation control the afferent input of nociceptive signals at the spinal level and the process can lead to either facilitate or inhibit the spinal transmission of pain to brain (Heinricher et al. 2009). Facilitation have been implicated in the form of chronic pain and several studies have documented the involvement of brainstem structures in the generation and maintenance of central sensitization and hyperalgesia (Zambreanu et al. 2005; Gebhart 2004). Impaired inhibitory modulation was reported in painful CP patients base on human model (Olesen et al. 2010a; Bouwense et al. 2013). In addition, brainstem facilitation was reported to maintain pancreatic pain in an animal model of CP (Vera-Portocarrero et al. 2006). Until today, no studies have attempted to modulate pain modulation in patients with CP, but emerging evidence from other chronic pain conditions suggest that selective serotonin–noradrenaline reuptake inhibitors (SNRIs) may be useful to augment descending inhibitory modulation and thereby to relieve pain (Yarnitsky et al. 2012). In Fig. 5.1 a schematic illustration of the different mechanisms is shown.

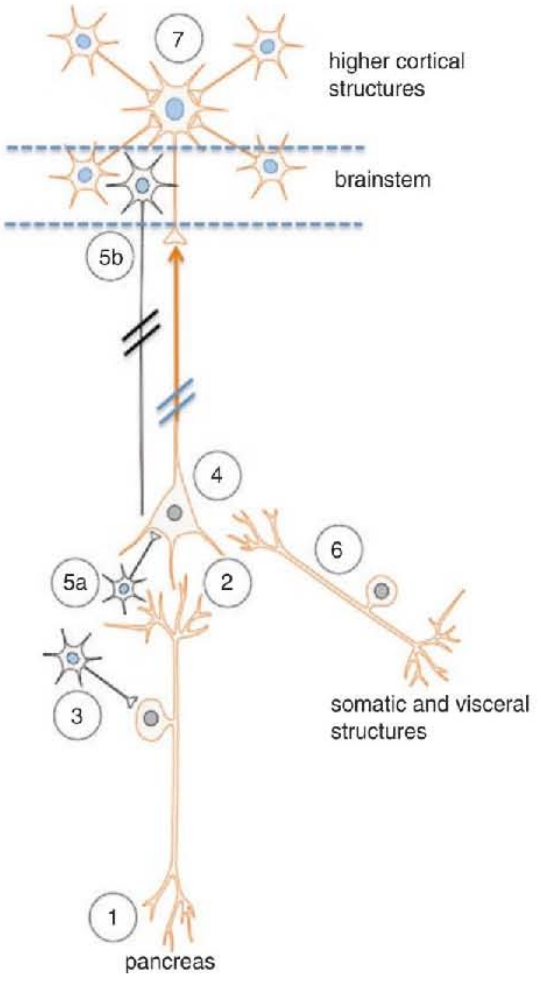

Fig. 5.1 Schematic illustration of the different nervous mechanisms thought to be involved in pancreatic pain. (1) Peripheral nerve damage with ectopic activity resulting in stimulus dependent and spontaneous pain; (2) Sprouting of non-nociceptive nerve afferents into "pain specific" areas of the spinal cord resulting in allodynia; (3) Sprouting of sympathetic neurons (*black*) into the dorsal horn neurons rendering the system sensitive to sympathetic activity and catecholamine; (4) Sensitization and phenotypic changes of spinal neurons due to the increased afferent barrage; (5) Defects in the normal inhibition from (a) interneurons and (b) descending tracts arising in the brainstem (*black*); (6) Abnormal coding of the afferent input from somatic areas and other viscera resulting in increased referred pain and viscero-visceral hyperalgesia; (7) Reorganisation and structural changes in the brain that encodes complex sensations such as affective, evaluative and cognitive responses to pain

Are Changes in Central Pain Processing Depending on a Nociceptive Input from the Pancreatic Nerves?

As can be seen from the above sections, several lines of evidence indicate that central pain processing is abnormal in CP. However, from the current evidence it is difficult to determine whether these central abnormalities depend on a nociceptive input from the pancreatic nerves (Gebhart 2007). There is support from other diseases such as in peripheral nerve injury and painful polyneuropathy that regardless of signs of central sensitization, primary afferent input is critical for maintaining on going and evoked neuropathic pain (Haroutounian et al. 2014; Vaso et al. 2014). The efficacy of topically applied drugs in these conditions also supports peripheral pain-generating mechanisms (Backonja et al. 2008; Meier et al. 2003). A small cross-sectional study in CP patients found that in hyperalgesic patients the generation of pain was independent of the pancreatic nociceptive drive and consequently denervation of pancreatic nerves was ineffective (Bouwense et al. 2011b). However, larger and longer-term studies that include systematic evaluation of the pain system prior and after intervention are still needed for confirmation (Table 5.2).

Table 5.2 Central pain mechanisms in chronic pancreatitis and associated experimental and clinical manifestations

Mechanism	Experimental evidence and clinical manifestations
Central sensitization	Hyperalgesia
	Allodynia
	Expansion of referred pain area (pancreatic viscerotome—Th10)
Abnormal patterns of brain activity	Functional reorganization of the visceral pain matrix
	Increased cortical excitability
	Abnormal brain rhythmicity (increased theta activity)
Structural alterations of brain morphology	Changes in microstructure of the brain
	Cortical thinning
Impaired descending pain modulation	Blunted CPM response

1.3 Pancreatic and Extra-Pancreatic Complications

In addition to the "plumbing" and "wiring" problems discussed above, many patients with CP experience pain due to intra- and extra-pancreatic complications of the disease. These are often easy to diagnose and treat and should always be considered when the patient is experiencing an exacerbation in pain symptoms. Among many, the most common are listed below.

1.3.1 Pseudocysts

As a relatively common complication, the estimated incidence of pancreatic pseudocysts is 20–40% (Boerma et al. 2000; Andrén-Sandberg and Dervenis 2004). Although lacking of long term follow-up studies, due to the chronic nature course of the disease, CP patients are at high risk of developing pseudocyst (Ammann et al. 1984). However, it is important to identify whether pseudocysts are asymptomatic or not according to the etiology, localization and size, the most influential factor of pain (Aghdassi et al. 2008; Gouyon et al. 1997).

1.3.2 Duodenal and Bile Duct Obstruction

The clinical presentation of duodenal and bile duct obstruction secondary to CP can be from asymptomatic to variable as postprandial/upper abdominal pain, early satiety, nausea and potential vomiting, fever, jaundice (Vijungco and Prinz 2003; Kalvaria et al. 1989; Prinz et al. 1985). It is reported that without cholangitis bile duct obstruction does not cause pain and the relationship between "obstructive pain" and pain in patients with CP is still unclear (Kahl et al. 2004).

1.3.3 Peptic Ulcer

Previous studies have demonstrated that the prevalence of duodenal ulcer is high in patients with CP (ranges from 3.6 to 37.5%) and upper abdominal pain due to peptic ulcer can be mistaken as pancreatic pain (Lankisch et al. 1993; Chebli et al. 2002; Schulze et al. 1983). It is suggested that the high prevalence of peptic ulcer can be attributed to higher infection rate of *Helicobacter*

pylori (Kalvaria et al. 1989), increased gastric acid secretion (Saunders et al. 1978; Piubello et al. 1982), and decreased bicarbonate secretion and duodenal pH due to pancreatic exocrine insufficiency (Brock et al. 2012). Moreover, recurrent acute pancreatic attack may redistribute gastric and intestinal blood flow.

As peptic ulcer can also be asymptomatic and patients with CP are more likely to undergo an diagnostic or therapeutic upper gastrointestinal endoscopy, the high prevalence may also can be a result of a "detection bias" (Schulze et al. 1983).

1.4 Side Effects to Treatment

While strong opioids are effective to relieve pain in CP patients, opioids frequently result in gastrointestinal side effects, including constipation, reflux, nausea and abdominal pain (Brock et al. 2012). Chronic abdominal pain was reported to be 58% in patients treated with opioids for non-cancerous diseases (Tuteja et al. 2010). Other medications that affect bowel motility and disturbed GI motor function due to exocrine pancreatic insufficiency may also indirectly contribute to the bacterial overgrowth that is reported in up to 40% of the patients and may result in abdominal distension and pain (Layer 1995; Casellas et al. 1998).

Complications to surgical and endoscopic therapy can also result in abdominal pain. However, no studies have examined the relative contribution to abdominal pain due to surgical complications in CP.

1.5 Conclusion

The pain mechanisms in chronic pancreatitis are heterogeneous and multifaceted, and likely often overlap and co-exist in the individual patient. Whereas the focus of pain treatment previously targeted abnormal anatomical findings such as strictures and stones in the main pancreatic duct there is little evidence to support that this is the reason for pain. Rather, neuronal changes in the peripheral and central nervous system are the main reasons for pain in most patients and often complicated further by cognitive and emotional distress. Therefore, the treatment should be multidisciplinary and based on a thorough workup of the pain system and in some cases combined with a psychological evaluation. It is mandatory to exclude secondary causes of pain, including side effects to treatment, as they are often reversible and easy to treat. As the combination of pain mechanisms in each patient is unique with distinct causes of pain and response to treatment, personalised treatment based on biomarkers that reflects the pain processing is an unmet need that invariably will be the focus for future studies.

2 The Mechanism of Pancreatic Stone Formation

Bo Ye and Wei-Qin Li

2.1 Introduction

Pancreatic stone is one of the features of chronic pancreatitis (CP) (Figs. 5.2 and 5.3), and almost 90% patients had pancreatic stones in the course

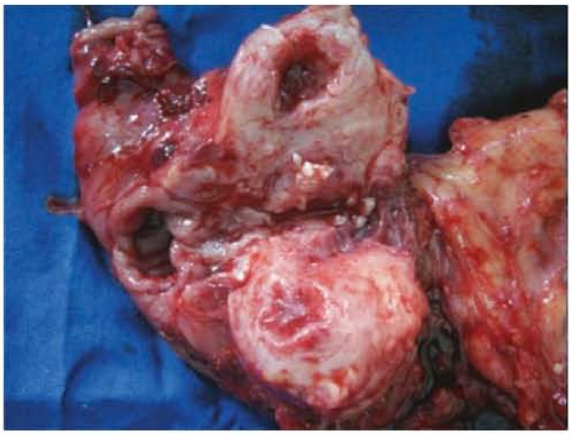

Fig. 5.2 Pancreatic stones in human surgical specimens. Reprint with permission from shanghai science & technology press

Fig. 5.3 Pancreatic stones in human fecal. Reprint with permission from shanghai science & technology press

of CP (Mariani et al. 1991). Researches on the composition and formation of pancreatic stones will help to understand the pathophysiological process of CP.

The composition of pancreatic stone is almost clear. Outer region of pancreatic stones consists of calcium carbonate as a major component, while the inner part (nidus) is comprised of a very fine network of fibers which are composed of proteins and glycosaminoglycans. The possible related mechanisms included the decreasing secretion of pancreatic stone protein, much greater concentration of lactoferrin in pancreatic juice, precipitating of trypsin in early stage, overexpressing of osteopontin and GP-2 in human pancreatic juice.

2.2 Analysis of Chemical Composition in Pancreatic Stones

2.2.1 Chemical Elements in Pancreatic Stones

Calcium is the most abundant chemical element in pancreatic stones (almost 90%). Other elements such as P, S, K, Fe, Cr, Ni were also found. Pitchumoni et al. (1987) studied the major, minor, and trace elements present in pancreatic stones by DC-plasma emission (DCP) spectroscopy and found that pancreatic stones were made up of calcite and contained, in addition to calcium, 17 other elements. They also studied the morphology, nature, and elements in human pancreatic stones by scanning electron microscopy (SEM) and energy dispersive X-ray fluorescence (EDXRF) and found that the amorphous nidus only contained iron, chromium, and nickel, whereas the outer shell was made up of calcium and 17 other elements.

2.2.2 Composition of Human Pancreatic Stones

It is important to know how the organic and inorganic components interact with each other during pancreatic stone formation. Calcium carbonate was the major inorganic component in pancreatic stones. The organic matrix of pancreatic stones includes proteins and glycosaminoglycans (mucopolysaccharides and mucosubstances). Comparing pancreatic stones before and after decalcification, Bockman et al. (1986) found carbonate more common on rounded,

lamellar, or otherwise modified surfaces by scanning electron microscopy. They were embedded in a gel-like matrix. Histochemical studies found that the gel-like matrix was made up of acid glycosaminoglycans, acid glycoprotein, and neutral glycoprotein. Farnbacher et al. (2005) found that clogging material in pancreatic stents contained mucopolysaccharides, crystals, and plant material, as well as visible calcium carbonate calculi.

2.3 Mechanisms of Pancreatic Stones Formation

Pancreatic stones are, in fact, intraductal calculi. According to the locations of stones, pancreatic stones mainly divided into main pancreatic duct and branch duct stones. Calculi in main pancreatic ducts is more common in the tropical chronic pancreatitis (TCP), while alcoholic chronic pancreatitis (ACP) is mostly characterized by the formation in pancreatic branch ducts of calculi (Pitchumoni 1984). Pancreatic stone also includes the precalcified forms and the calcified forms. However, the mechanisms of different forms of pancreatic stone formation are almost the same, including protein precipitation and increased $CaCO_3$ crystal growth in pancreatic juice. Nevertheless, it is still not clear that which protein is secreted abnormally causing protein precipitation. Current theories or hypothesis involved mainly were as follows.

2.3.1 Pancreatic Stone Protein

Human pancreatic stone protein (PSP) is one of the regeneration (reg) gene proteins, which is located on the short arm of chromosome 2 in 2p12 spans. There are five immunoreactive forms PSP S1-5 detected in pancreatic juice. PSP S2-5 corresponds to the four isoforms distinguishable by SDS-PAGE (Stewart 1989). It was believed to inhibit spontaneous calcium carbonate precipitation from highly supersaturated solutions and was called "lithostathine" by some. PSP was detectable in almost all pancreatic stones, ranging widely from only a trace amount to 1.21% as a percentage of the stone weight. A wide range of percentages (ranging from 0.01 to 41.9%) of pancreatic stone protein in the total protein suggests that the mechanisms and protein components involved in the stone formation are multifactorial (Jin et al. 2002). Current hypothesis about PSP included stabilizing pancreatic juice hypothesis, calcium binding hypothesis and adsorption hypothesis.

Stabilizing Pancreatic Juice Hypothesis

Sarles and Bernard (1991) suggested that PSP would inhibit calcite crystal nucleation and growth in the pancreatic juice to prevent pancreatic stone formation. Bimmler et al. (1997) produced rat PSP in a baculovirus expression system and confirmed its calcite crystal inhibitor activity. They also found several organic and inorganic components of pancreatic juice (trypsinogen, phosphate) which had inhibitory activity on calcium carbonate crystal formation. Addadi and Weiner (1985) also reported PSP had an unspecific inhibition of calcite crystal growth. However, De Reggi et al. (1998) proposed that the inhibitory property was dut to a high concentration of Tris buffer. Therefore, PSP might affect stone formation, but the debate on lithostathine function remains.

Calcium Binding Hypothesis

Lohse and Kraemer (1984) found PSP had four equivalent and independent calcium binding sites by using radioactive ^{45}Ca in equilibrium dialysis experiments. They thought that calcium binding can modify the physico-chemical characteristics of PSP, result in the formation of protein plugs, which could illustrate the presence of the protein in pancreatic stones. However, Multigner et al. (1986) observed the absence of calcium in the proteic core of some stones. Therefore, calcium binding sites on PSP may

explain its inhibition of calcite nucleation, precipitation and crystal growth.

Adsorption Hypothesis

Gross and Caro (Gross et al. 1985; De Caro et al. 1979) found that PSP had a greater affinity for the crystal. Sarles and Bernard (1991) thought that PSP can adsorb on crystal surface. Geider et al. (1996) proposed that the adsorption of PSP to the crystal resulted in the modification of the crystal shape. De Reggi et al. (1998) observed that the quantities of adsorbed PSP and albumin per unit of surface were in the same range and the adsorption of PSP on calcite was not much higher than for an amorphous phase (glass). Therefore, the adsorption of PSP on calcite does not interact specifically.

2.3.2 Lactoferrin

Lactoferrin (LF) is a globular glycoprotein 80 kDa, which is widely represented in tears, saliva, nasal secretions and other secretory fluids. Which is also present in PMN. And some acinar cells also secret it. It also has many immunoregulatory effects including antimicrobial activity (bacteriocide, fungicide) and innate defense.

The concentration of Lactoferrin tends to increase in CP. Hayakawa et al. (1983) found the concentration of LF was much higher the protein plugs by analyzing the pancreatic stones obtained from the 13 patients with chronic calcified pancreatitis (Nagai and Ohtsubo 1984). This suggested that lactoferrin may play an important role in early stage of protein plug formation in the pancreatic duct.

2.3.3 Trypsinogen

Trypsinogen is found in pancreatic juice, which is the precursor form or zymogen of trypsin. Once it is activated by enteropeptidase, the trypsin can activate more trypsinogen into trypsin. Allan and White (1974) found intraductal pancreatic zymogens were activated in some patients with CP. Hayakawa et al. (1994) observed the immunoreactivity of human cationic trypsin in protein extracts from pancreatic stones in CP patients ranged from 0 to 42.3 ng/μg protein. They also observed that more densely immunoreactivity was presented in the center of the stones than in the concentric laminar layer of the periphery by using an immunogold technic SEM. Tympner (1981) found trypsin activity increased in pure pancreatic juice aspirated from CP patients. Renner et al. (1980) also found trypsinogen increased highly in in the pure pancreatic secretions in ACP patients. Therefore, we can concluded that hyperconcentration of pancreatic zymogens and proteins and activation of the trypsinogen were associated with pancreatic stone formation in early stage.

2.3.4 Osteopontin

Osteopontin is a 44-kDa glycosylated phosphoprotein, which is one of a group of noncollagenous bone matrix components. Recently, it has been reported that there is a relation between osteopontin expression and several diseases associated with calcification, such as atherosclerosis, breast cancer, meningioma and urinary stone formation.

Nakamura et al. (2002) found that osteopontin mRNA was detected in CP but not in normal pancreas specimens. In situ RT-PCR, they revealed that osteopontin was expressed in acinar or ductal cells in all 11 CCP patients, whereas in 5 of 9 CP cases without pancreatic stones osteopontin was not expressed in acinar or ductal cells. Therefore, acinar cells and ductal cells may secrete osteopontin, which can bind to protein plague and may play a crucial role in in pancreatic stone formation.

2.3.5 GP-2

GP-2 (glycoprotein-2) is the most abundant protein of the zymogen granule membrane of the exocrine pancreas, which is linked to the membrane through a glycophophatidyl inositol (GPI) bond (Colomer et al. 1994). GP-2 was also shown to be homologous to Tamm-Horsfall protein, a GPI linked protein produced in kidney and

excreted in urine, which is the major component of hyaline casts found in urine.

GP-2 is present in pancreatic juice and makes up 5–8% of unstimulated juice protein in the rat and pig. Stimulation with secretin has no effect on GP-2 output in pancreatic juice while stimulation with caerulein or carbachol increases secretion, but with a slower time course than the secretion of digestive enzymes. GP-2 is also present in ductal proteinaceous plugs found in chronic pancreatitis Freedman et al. (1994) examined the protein composition of intraductal plugs from patients with noncalcific chronic pancreatitis by SDS-PAGE and found GP-2 were both a reproducible constituent and enriched within intraductal plugs. Therefore, GP2 may play a role in pancreatic plug formation, which is an important step in pancreatic stone formation.

2.4 Conclusion

Study on pancreatic stone composition and its formation mechanism is ultimately able to give more intervention and treatment in early stage of CP. Noda and Tsujimoto et al. (Noda et al. 1997; Tsujimoto et al. 2005) found that bromhexine hydrochloride had a high affinity for the pancreas, acting directly on the mucus-producing cells and causing them to produce low-viscosity mucus. Lohse et al. (Lohse et al. 1981; Noda et al. 1984) observed that organic acids dissolved pancreatic stones by chelating with calcium ions. Therefore, further work is needed to identify agents that dissolve protein plugs and pancreatic stones, which may have potential to eliminate pancreatic duct obstruction to relieve pancreatitis attacks and pancreatic pain.

In conclusion, the mechanisms of pancreatic stone formation are complicated, and many proteins may play a role in the protein precipitation. So far, protein plugs formation and precipitation of $CaCO_3$ crystal are the most important two factors in pancreatic stone formation.

3 Experimental Models of Chronic Pancreatitis

Ali A. Aghdassi, Matthias Sendler, Julia Mayerle, and Markus M. Lerch

Chronic pancreatitis is an inflammatory disorder of the pancreatic gland. In western countries its incidence is reported to be between 4 and 13/100,000, which is somewhat lower compared to acute pancreatitis with an estimated incidence of 13–45/100,000 persons (Levy et al. 2014; Yadav and Lowenfels 2013). However, according to recent population based studies incidence rates of chronic pancreatitis have steadily increased during the last decades and showed regional differences (Yadav and Lowenfels 2013; Yadav et al. 2011).

From epidemiological data and clinical experience we know that chronic pancreatitis can develop from either recurrent bouts of acute pancreatitis in which the pancreas does not recover completely from the injury to the organ. About 20–30% of patients with acute pancreatitis suffer from recurrences and about 10% develop chronic pancreatitis. The presence of continuous environmental exposure (alcohol, tobacco) or genetic risk factors favor progression to chronic pancreatitis (Yadav and Lowenfels 2013; Yadav et al. 2012). To a much lesser extent chronic pancreatitis can develop as a result of one severe attack of acute pancreatitis that leads to necrosis and fibrosis directly (Lankisch et al. 2009).

3.1 Features of Chronic Pancreatitis

Both morphological changes and clinical symptoms characterize chronic pancreatitis and their knowledge is of importance for a comprehensive understanding of the available experimental models with their strengths and limitations. So

far there is no animal model that incorporates *all* features of chronic pancreatitis that are seen in humans. Secondly, a variety of animal models exist that, although mimicking chronic pancreatitis, differ among each other regarding their morphologic and clinical characteristics. Choosing the right models is of importance and depends on the scientific question that is addressed by the investigator.

From a histopathologic point of view chronic pancreatitis is a progressive and destructive inflammatory process of the pancreatic parenchyma that finally involves ductal changes as well. In some cases the kind of histologic damage allows a deduction of the etiology of chronic pancreatitis, as some causative factors show typical histologic features (Klöppel 2013; Kloppel et al. 2004). Generally chronic pancreatitis starts with focal necrosis and inflammatory cell invasion. When pancreatic stellate cells are activated collagen deposition occurs leading to fibrosis of the organ. In early stages and particularly in alcoholic chronic pancreatitis fibrosis is mainly localized in a perilobular pattern that later affects the parenchyma (intralobular fibrosis) (Klöppel 2013). In late stages ductal changes with irregularities of the lumen width and strictures are seen that are the result of tissue traction due to fibrosis and scarring. Ductal changes ultimately predispose to precipitation of calculi and protein plugs (Ammann et al. 1996; Detlefsen et al. 2006; Klöppel 2007).

Typical clinical manifestations are pancreatic pain, steatorrhoea with weight loss and maldigestion due to exocrine insufficiency and impaired glucose tolerance up to manifest diabetes mellitus (Muniraj et al. 2014; Schütte et al. 2013). Continuous maldigestion without medical intervention leads to malnutrition with additional complications such as osteoporosis and vitamin deficiency (Gupte and Forsmark 2014). Signs of exocrine insufficiency occur late when secretory function is reduced to less than 10% of normal (Keller et al. 2009).

3.2 Animal Models for Chronic Pancreatitis

Experimental models for chronic pancreatitis have been established in many animal species using various techniques (Fig. 5.4). Most of them were adapted to rodents. Chronic pancreatitis models can be classified either according to their mechanism of induction, that are meant to resemble the pathophysiology in humans or based on a completely different mechanism (Aghdassi et al. 2011; Lerch and Gorelick 2013), or, alternatively, according to their morphological and clinico-biochemical characteristics. The aim of this section is to introduce frequently used animal models for experimental chronic pancreatitis including their strengths and shortcomings. We also want to point out recent developments in genetic models of chronic pancreatitis that attracted increasing attention. Furthermore, we specify what kind of pathology is seen in each model and whether it can be used for assessment of either morphological or clinical characteristics of chronic pancreatitis or both.

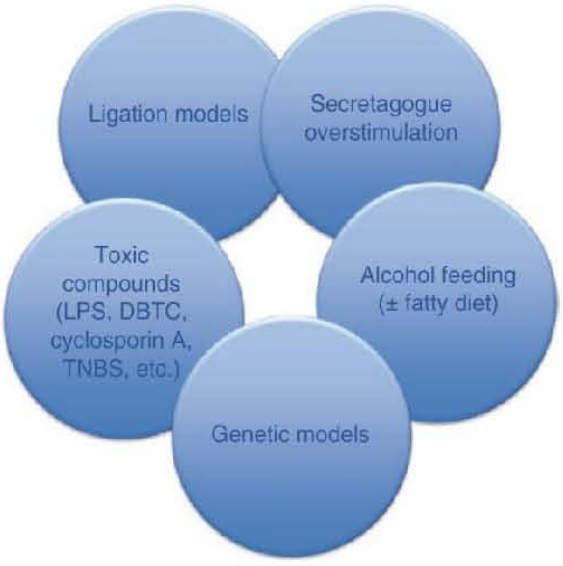

Fig. 5.4 In-vivo experimental models for chronic pancreatitis. Techniques have been applied alone or in combination

3.3 Duct Obstruction Models

Many studies have demonstrated that obstruction of the pancreatic duct, either partial or complete, leads to morphological changes compatible with chronic pancreatitis. Proximal to the stenosis intraductal pressure increases and favors duct dilation with atrophy of acinar cells and replacement by fibrous tissue. In humans possible causes for a blockage of the duct are tumors (either adenocarcinoma or cystic or endocrine neoplasms) or more rarely cystic fibrosis that plugs the ductal lumen by its viscous mucin (Kloppel et al. 2004). Humans have one main pancreatic duct that enters the duodenum at the major duodenal papilla after joining the bile duct. Early in embryonic development two efferent pancreatic ducts, which origin form the ventral and dorsal part of the organ, fuse together to form one main duct. If this fusion fails to occur (in up to 9% of autopsy studies) two separate pancreatic ducts are preserved, a condition known as pancreas divisum (Brock et al. 2013).

3.3.1 Pancreatic Anatomy in Rodents

Currently the majority of pancreatic duct obstruction models are performed in rodents. However, the anatomy of the pancreas differs among species. Since most studies are carried out in mice or rats we want to give more detailed information regarding anatomy of the pancreas in these two species. Compared to the human pancreas in which gross anatomy shows a more compact or cohesive pattern the mouse pancreas is more loose as it consists of three separate lobes, the gastric, duodenal and the splenic lobe and is frequently interspersed with fatty, connective or lymphoid tissue (Treuting et al. 2012). Each lobe is drained by a separate pancreatic duct. Duct anatomy of the mouse pancreas shows variations that make ligation procedures more complex. Usually the ducts from the splenic and gastric lobe converge and will be completed by the duct from the duodenal lobe to form one common channel before opening into the common bile duct. In about 10% the splenic and the gastric duct join the duodenum separately with an accessory papilla (Watanabe et al. 1995). Like in mice the rat pancreas also consists of three lobes, two smaller ones (the biliary and the duodenal lobe) and a larger gastrosplenic lobe (Kara 2005). The pancreatic duct system consists of two main ducts, the anterior and the posterior pancreatic duct. Both finally open into the biliopancreatic duct. In addition minor ducts of all three lobes are petering out separately to the biliopancreatic duct (Kara 2005).

The size of pancreatic duct varies naturally between species. Magnetic resonance imaging (MRI) and autopsy studies revealed that the mouse pancreatic duct has an approximate diameter of 88 μm and a total tractable length of 5850 μm (Grippo et al. 2011). Caliber of the rat biliopancreatic duct is larger with around 1 mm and a length of ca. 29 mm (Kara 2005) so that ligation procedures are technically easier.

Selective obstruction of one branch of the pancreatic duct and not of the whole biliopancreatic duct uncouples only one part of the pancreas that will be injured. For this reason induction of chronic pancreatitis can be locally controlled within the pancreas. Since the unobstructed part remains unaffected it can serve as an internal control (Kishi et al. 2003; Sendler et al. 2015). Technique of (selective) ductal ligation forms a big advantage over other models of chronic pancreatitis as a "negative" control is created within the same animal ruling out confounding variables.

3.3.2 Ligation Models in Rodents

Ligation models belong to the best experimental models for mimicking chronic pancreatitis and are well established for mice and rats. In general first changes start within the first 7–10 days after the intervention with necrosis, an inflammatory cell infiltrate and first signs of acinar cell atrophy, ductal cell proliferation and initiation of fibrosis that is often visible with periductal and intralobular arrangement (Watanabe et al. 1995; Sendler et al. 2015; Churg and Richter 1971; Miyauchi et al. 2007; Scoggins et al. 2000; Yamamoto et al.

2006). Emergence of fibrosis is associated with activation of pancreatic stellate cells (PSCs) that transform from a quiescent to an active state by increased expression of alpha smooth muscle actin (αSMA) and deposition of extracellular matrix components (Apte et al. 1999). Later (≥14 days) changes include more extensive fibrosis with upregulation of collagen I and III production and a more perilobular distribution. In addition a fatty tissue replacement occurs (Watanabe et al. 1995; Sendler et al. 2015; Yamamoto et al. 2006).

Notably, exocrine insufficiency is hardly seen in the ligation models unless a high fat diet is fed. On the one hand this is caused by the incomplete obstruction of the pancreatic ductal system so that one part of the pancreas remains unaffected and keeps up secretory enzyme production. Secondly very early after ligation a subgroup of pancreatic cells, intermediate cells, start a transdifferentiation and display both exocrine and endocrine phenotypes as indicated by co-expression of insulin and amylase (Bertelli and Bendayan 1997). These cells are detectable within the islets of Langerhans and within exocrine tissue and indicate a high degree of plasticity of the organ within the first days after the insult to maintain exocrine and endocrine function.

The lack of endocrine insufficiency might be also explained by an increased islet beta-cell proliferation in duct-ligated rats. The Beta cell population nearly doubled within the first week after the ligation and small islets and islet-cell clusters developed (Wang et al. 1995). Interestingly, glucose transporter type 2 (GLUT-2), the major glucose transporter isoform, was expressed in ductal cells, besides its primary location on insulin secreting beta cells. Obviously ligation-stimulated ductal cells reach a metaplastic state and acquire properties of endocrine cells with higher glucose sensitivity (Wang et al. 1995).

3.3.3 Variations of Classical Ligation Procedures

A continuous pancreatic duct hypertension was induced by double-ligation of the bilio-pancreatic duct at its proximal and distal end in rats. Both pancreatic juice and bile were selective collected and diverted into the duodenum. Two weeks after the procedure mucoprotein concentration of the pancreatic juice was increased despite a decrease of digestive enzyme contents indicating that non-enzymatic protein secretion starts on sustained pancreatic duct hypertension (Yamamoto et al. 2006). Previous studies in humans already showed that non-enzymatic protein secretion leads to enhanced viscosity of the pancreatic juice and formation of intraluminal protein plugs (Harada et al. 1981). First histomorphologic changes were observed earlier, i.e. at already 7 days, with intralobular and perilobular fibrosis and a peak of acinar cell apoptosis.

Possibly the observation time needs to be much longer for studying effects on exocrine function. In a model established by Isaksson and collaborators the rat pancreatic duct was obstructed with a glue-like substance consisting of either acrylate or prolamine and animals were monitored for 5 months in total. Exocrine insufficiency was present as evidenced by reduced secretory enzyme levels whereas endocrine function was not impaired (Isaksson et al. 1983). These results also tell us that the investigation of long-term effects on the pancreas is technically possible when using duct occlusion models but an observation time of months will be required.

3.3.4 Ligation Models in Non-rodents

There are fewer studies dealing with mammals whose pancreatic anatomy looks more similar to the human one. In dogs ligation procedures were studied that led to typical morphological characteristics of chronic pancreatitis. These changes developed within 3–6 months. Pancreatic excretory capacity was diminished, too (Tanaka et al. 1988, 1998). Compared to rats connective tissue replacement was stronger, however acinar atrophy was less marked (Churg and Richter 1971). The degree of pancreatic damage was much more distinct, when ligation was performed in conjunction with chronic ischemia or ethanol administration to these dogs (Tanaka et al. 1988).

3.3.5 Limiting Factors of Ligation Models

Comparison of ligation models is limited as the location of ligation differs among studies. In addition other stimuli were applied to animals such as caerulein (Sendler et al. 2015), ischemia (Tanaka et al. 1988) or ethanol feeding (Tanaka et al. 1988) that affect severity of the disease. Knowledge of the exact location of pancreatic duct ligation is of high importance when comparing the extent of changes of chronic pancreatitis.

Ligation procedures are rather invasive procedure and demand surgical skills and exact knowledge on the anatomy of abdominal organs. Moreover technical equipment has to be available for doing the operation. These factors set limitations on general application of duct-ligations models. A learning curve when doing the procedure is inevitable and has to be taken into account when analyzing the results.

3.4 Repeated Caerulein Models

The secretagogue hyperstimulation model is by far the most frequently used technique for induction of acute pancreatitis. This method was first used by Lampel and Kern who intravenously applied caerulein, an ortholog of the intestinal hormone cholecystokinin, to rats and observed signs of acute interstitial pancreatitis (Lampel and Kern 1977). Pancreatitis usually resolves spontaneously. This model was broadly adopted and modified to secretagogue application given intraperitoneally which is as effective but less technically challenging. Usually the required dose exceeds tenfold the concentration needed for maximal physiological secretion from the exocrine pancreas. Later caerulein-injection models were modified again to establish experimental chronic pancreatitis following the idea that multiple bouts of acute pancreatitis finally lead to chronic disease (Lerch and Gorelick 2013). Normally pro-fibrogenic cytokines peak within 3 or 4 days after acute pancreatitis and normalize within 1 week but reinjury before normalization of the profibrogenic milieu favors chronic pancreatic injury. TGF-beta is believed to regulate extracellular matrix deposition as it is elevated during the vulnerable phase of acute pancreatitis and, when inhibited, collagen and fibronectin production are reduced (Gress et al. 1994; Menke et al. 1997). Increase of TGF-beta was not only observed in caerulein-induced acute pancreatitis but also in an obstruction model (duodenal loop closure) underlining its general importance during acute pancreatitis (Kimura et al. 1995).

Supraphysiologic concentrations of caerulein given two or three times a week for up to 6–10 weeks showed substantial pancreatic fibrosis in line with a strong increase of procollagen I expression (Neuschwander-Tetri et al. 2000a, b). Longer intervals of caerulein applications seemed to be no more effective. No or hardly any fibrosis was observed during weekly applications unless recombinant TGF-beta was given additionally (Van Laethem et al. 1996). When intervals were extended up to 20 days no sustainable signs of fibrosis were seen (Elsasser et al. 1992).

Although chronic fibrotic changes can be generated successfully by repeated caerulein applications it remains unclear whether exocrine or endocrine insufficiency can be achieved. Diabetes was observed in an experimental model in rats using caerulein injections plus water-immersion stress (Goto et al. 1995; Miyahara et al. 1999). Stress or caerulein alone did not cause endocrine insufficiency. The intrapancreatic protein content, reflecting exocrine function, was nearly halved when mice were treated with caerulein (three times a week) and lipolysaccharide for 6 weeks (Ohashi et al. 2006).

There are several other noxious agents that have been combined with caerulein hyperstimulation to induce experimental chronic pancreatitis. Some of them will be introduced in the following sections.

3.5 Alcohol Feeding Models

Undoubtedly alcohol consumption increases the risk of acute and recurrent acute pancreatitis and it is the single most frequent cause for chronic pancreatitis in humans (Yadav and Lowenfels 2013; Aghdassi et al. 2015). Although there is no clear threshold of an alcohol amount that inevitably leads to pancreatic fibrosis heavy alcohol consumption is associated with a higher risk of developing chronic pancreatitis (Frulloni et al. 2009; Irving et al. 2009). The harmful effects of alcohol have been explored in several studies using whole animal or ex-vivo models and there are plausible data on the pathophysiologic way in which ethanol injures the pancreas (Gukovskaya et al. 2006; Pfutzer et al. 2002). Alcohol is metabolized either in an oxidative pathway by the successive action of alcohol-dehydrogenases (ADHs) and aldehyde-dehydrogenases (ALDHs) into its metabolites acetaldehyde and acetate. In the non-oxidative pathway ethanol is combined with fatty acids to fatty ethyl esters (FAEEs) (Criddle 2015). Both pathways occur in the pancreas and both ethanol and its catabolic end-products are toxic to pancreatic cells. On acinar cells they directly induce cellular and organellar (zymogen granules, mitochondria) injury that leads to intracellular protease activation and cell death. As a consequence cytokines are released and inflammatory cells are attracted aggravating the damage. Besides the effect on acinar cells ethanol and metabolites effect on pancreatic stellate cells (PSCs) causing synthesis of extracellular matrix components (Apte et al. 2000; Lugea et al. 2003).

It has attempted to translate the clinical features associated with alcohol consumption into experimental animal models for chronic pancreatitis. Unfortunately the results are very disappointing and so far there is no satisfactory model for chronic pancreatitis using ethanol application alone. Besides the pancreas other organs such as lung and liver are injured by alcohol exposure and animals don't develop proper chronic pancreatitis (Li et al. 2008; McIlwrath and Westlund 2015; Schneider et al. 2009).

3.5.1 Duration of Alcohol Application

Technically it is possible to induce chronic pancreatitis in animals with alcohol. Usually alcohol is given together with their daily diet via an oral route often as a Lieber-DeCarli liquid diet. The concentration of ingested ethanol can vary (see below). In more uncommon experimental setups alcohol is applied by other ways, i.e. via a gastrostomy or directly into the pancreatic duct.

Features of chronic pancreatitis such as acinar cell atrophy and fibrous tissue replacement were achieved by long-term ethanol feeding (up to 30 months) in rats. Pattern of fibrosis resembled those of human chronic pancreatitis (segmental or perilobular distribution) and intraductal protein plugs with partial calcifications were seen as known from humans. Signs of chronic pancreatitis appeared very late when only alcohol is applied and were noticed between the 20th and 30th month of alcohol exposure. By that time already half of the animals died of old age (Sarles et al. 1971). It is quite clear that application of alcohol alone imposes limitations, as long-term treatment and a high death rate are not only time consuming but also questionable from an ethical pathophysiology point of view.

In more recently developed experimental models shorter treatment periods (up to 6 months) were used. However, morphologic changes were less distinct. Secretory enzyme levels behaved differently under chronic ethanol exposure: Amylase secretion was impaired but levels of zymogens such as pro-elastase and (chymo-)trypsinogen were enhanced something normally observed during acute pancreatitis (Li et al. 2008; Perkins et al. 1995). When ethanol was injected directly into the pancreatic duct of rats histologic features of chronic disease like interstitial fibrosis, loss of exocrine acinar cells and ductal dilation were observed at quite early

time points (7 days). Limiting factors are the invasive technique and the unphysiologic route of alcohol administration to the animals (Unal et al. 2015).

3.5.2 Techniques of Alcohol Application to Animals

Alcohol supplementation in nutrition needs to exceed a minimum amount so that blood alcohol reaches toxic concentrations in the pancreas. Charles Lieber and Elonore DeCarli contributed much to the development of optimal nutrition formulas for scientific use when they investigated different liquid diets containing ethanol and their effects on organs. The natural aversion of rats against alcohol has been overcome by incorporation of alcohol in a fluid. Usually liquid diets can be prepared more easily than solid foods and are more flexible to adjust depending on the experimental design and the investigator's need. Controls can be generated by replacement of alcohol with other macronutrients such as carbohydrates (Lieber and DeCarli 1989; Lieber et al. 1965). Alcohol concentration in liquids can be calculated according to their proportion of energy supply for total energy intake. In rats an ethanol amount of 5 g/dL or 36% of total energy supply was found to achieve a reasonable blood concentration of at least 20 mM or 100 mg/dL. Lower blood alcohol levels would be ineffective and would not fit to real clinical condition. Higher alcohol concentrations usually will not be consumed by the rat because they dislike its taste (Lieber and DeCarli 1989). Meanwhile Lieber-DeCarli diets have gained wide acceptance as a standard for scientific use when studying effects of chronic alcohol abuse (Perkins et al. 1995).

There are alternative routes such as an application via a gastrostomy or gavage or directly into the ductal system after laparotomy that allows ingestion of high-percentage ethanol diets (Tsukamoto et al. 1988) but it remains debatable whether this type of application interferes too much with physiologic intrinsic or extrinsic stimulation of the pancreas. In addition unphysiologic application routes won't mimic human situation and pancreatic injury can arise due to other factors rather than ethanol (Lugea et al. 2010).

Gender of animals affects severity of alcohol-induced injury and therefore should be considered when designing an experiment. Alcohol induced liver injury is greater in females than in males demonstrated by histology and levels of serum transaminases (Iimuro et al. 1997). No direct comparative studies have been performed for evaluation of pancreatic damage so far. When using female rats for experiments pancreatic damage under high-dose ethanol and fat diet occurs earlier at already 8 weeks observation time, indicating that female animals might be more susceptible to pancreatic damage (Kono et al. 2001).

3.5.3 Alcohol Application and Fat

Only a minority of alcoholics (around 5%) ultimately develops chronic pancreatitis so that other co-factors need to be present for manifestation of overt disease. A majority of patients suffered from recurrent acute pancreatitis (RAP) before they come down with chronic pancreatitis. Time to progression varies and lies between 1 and 19 years (median 5.7 years) (Ammann et al. 1994). Alcohol is regarded to be a kind of *predisposing* or *sensitizing* agent to the pancreas but other triggers need to be present so that chronic pancreatic will develop (Aghdassi et al. 2015; Pandol et al. 2011).

One of them is fat. Dietary fat is an important contributor to alcohol-induced pancreatic injury. In the Tsukamoto-French enteral alcohol feeding model rats were fed by a liquid diet containing ethanol and different concentrations of fat. Fat was prepared from corn oil, that is mainly composed of (poly-)unsaturated fatty acids. Diets were classified as low-fat (4–5% of total calory intake), high-fat (22–25%) or extra high-fat (30–35%) diet (Tsukamoto et al. 1988). With increase of dietary fat content there was a potentiation of pancreatic injury: Histopathologic changes included acinar atrophy, a patchy distribution of

interstitial fibrosis, fatty replacement and some fat necrosis starting at 4 weeks of diet. Exocrine and endocrine function seemed to be not substantially altered, as plasma trypsinogen and glucose of the "high-fat" dietary group were comparable to controls. Results of high-fat diet were confirmed by further studies and some of them could even show impairment of endocrine function (McIlwrath and Westlund 2015).

Other routes of combined ethanol and fat administration have been tried. Direct intraperitoenal application of fatty acids and ethanol induced acute pancreatitis in a mouse model. Conversely, co-application of 3-benzyl-6-chloro-2-pyrone (3-BCP), an inhibitor of carboxy ester lipase (CEL), inhibited FAEE production and ameliorated pancreatic damage (Huang et al. 2014).

These results first indicate that a balance of oxidative and non-oxidative metabolization is critical for prevention of pancreatic damage. Preponderance of non-oxidative ethanol metabolism produces FAEEs and both ethanol and fatty acids exert toxic effects on the pancreas. An exogenous administration of fatty acids increases FAEE amounts and thus pancreatic damage. Secondly carboxy ester lipase (CEL) was identified to be one of the enzymes responsible for the damage. Notably, long-term ethanol feeding even increases pancreatic CEL activity (Pfutzer et al. 2002; Criddle 2015). On a cellular level FAEEs induced sustained high Ca^{2+} elevations that lead to uncontrolled trypsinogen activation (Gerasimenko et al. 2009). Moreover there is a loss of mitochondrial function by opening the mitochondrial transition pore leading to a fall of intracellular ATP levels that is a prerequisite for the emergence of necrosis (Criddle et al. 2006).

Pancreatic injury that is caused by high doses of ethanol and a fatty diet is less severe when medium-chain triglycerides, i.e. saturated fatty acids, are used instead of unsaturated fats (Kono et al. 2001). Less steatosis of the pancreas as well as reduced inflammatory infiltration and necrosis were observed. One of the underlying mechanisms might be a reduction of lipid peroxidation and consecutively a prevention of free radical formation in presence of MCT-fats.

3.5.4 Ethanol, Cholecystokinin and Chronic Pancreatitis

From ex-vivo experiments with isolated acinar cells and animal models it is well known that acute pancreatitis can be induced by repetitive applications of supraphysiologic doses of cholinergic and cholecystokinin (CCK) agonists in rodents (Lugea et al. 2010; Halangk et al. 2000). Therefore combinations of alcohol feeding with caerulein injections either as a single shot or by repetitive applications were thought to be an attractive way for induction of chronic pancreatitis (Lugea et al. 2010; Deng et al. 2005; Gukovsky et al. 2008; Perides et al. 2005). Usually ethanol feeding is performed prior to caerulein applications and lasts for approximately 2–8 weeks. Results consistently showed histopathologic changes compatible with chronic pancreatitis such as activation of pancreatic stellate cells with an increase of collagen content and fibrosis. Combination of alcohol and caerulein clearly enhanced fibrosis formation whereas alcohol alone only led to an inflammatory reaction (Deng et al. 2005; Perides et al. 2005). Exocrine insufficiency was not observed.

Usually a concentration of 50 µg/kg body weight is used for induction of acute pancreatitis but this dose can be markedly reduced (up to 0.5 µg/kg) when animals have been fed with an ethanol-diet before. This drastic reduction again underlines the ethanol sensitizing effect for other harmful stimuli.

3.5.5 Combination of Ethanol and Other Agents

Further agents have been used in combination with ethanol with promising results. Lipopolysaccharides (LPS) are known as endotoxins, cause activation of pancreatic stellate cells and inhibit apoptosis of PSCs so that extracellular matrix proteins will be increasingly

synthesized and released. Mice fed with ethanol and subjected to (intraperitoneal) LPS injections showed morphological signs of chronic pancreatitis whereas ethanol alone was ineffective (Nakayama et al. 2014). Changes were detectable after 6 weeks of LPS treatment. Intraductal bile salt infusions to rats that received both a Lieber DeCarli diet and caerulein injections developed severe necrotizing pancreatitis with concomitant lung injury (Schneider et al. 2009).

Cyclosporin A is an immunosuppressant that is clinically used for suppression of organ transplant rejection. Simultaneous treatment of rats with caerulein and daily cyclosporin led to acute pancreatitis without any regeneration of the organ. This distortion of the repair mechanism caused myofibroblast proliferation, collagen production and fibrosis as seen in chronic pancreatitis (Vaquero et al. 1999). In a modified experimental model when rats were pre-fed with an ethanol containing Lieber DeCarli diet (36% of total calorie intake) pancreatic damage was aggravated with enhanced fibrosis formation, acinar tissue loss and sustained inflammatory infiltration (Gukovsky et al. 2008). It is still unclear whether these models will be useful for investigation of exocrine or endocrine deficiencies, as well.

In other experimental settings alcohol feeding was performed *after* preceding interventions. Trinitrobenzene sulfonic acid (TNBS) is a chemical hapten that binds to tissue proteins capable of inducing T-cell mediated immunity and generation of oxygen radicals and other inflammatory mediators (Tatsumi and Lichtenberger 1996). This compound has already been used in rats for experimental models of colitis and cholangitis. A retrograde instillation of TNBS into the pancreatic ductal system causes fibrotic change of the organ and continuous weight loss. Endocrine insufficiency was not observed (Puig-Divi et al. 1996). When rats were fed with ethanol for another 2–4 weeks fibrosis and glandular atrophy were more pronounced and animals now showed an impaired glucose tolerance indicating beginning of endocrine insufficiency (Puig-Divi et al. 1999).

3.6 Genetic Models

From human studies there is increasing evidence that genetics plays an important role in the susceptibility to recurrent acute or chronic pancreatitis. Linkage and candidate gene analysis have discovered six major genes that target either acinar cells in a trypsin-dependent pathway (PRSS1, PRSS2, CTRC, CASR, SPINK1) or ductal cells (CFTR) and their mutations finally lead to loss or gain of function of the proteins (Aghdassi et al. 2015; Whitcomb 2012). In a much broader approach recent studies investigated genetic variants using genomewide association studies (GWAS) that discovered polymorphism in genes, which have not been associated with pancreatitis yet (Derikx et al. 2015; Weiss et al. 2015; Whitcomb et al. 2012).

3.6.1 Trypsinogen

PRSS1 gain of function mutations, such as p.R122H, are known to increase the risk of recurrent acute and chronic pancreatitis in humans (Whitcomb et al. 1996). This amino acid exchange renders trypsin to be more resistant to degradation. Meanwhile more PRSS1 mutations were identified in association with hereditary pancreatitis. For these reasons a transfer of clinical findings from bedside to bench, i.e. an animal model became attractive: Indeed, a transgenic mouse carrying the PRSS1 mutant R122H, placed under the control of an elastase promoter showed signs of chronic pancreatic disease. Fibrosis and acinar cell degeneration were observed. Changes started at 7 weeks of age and progressed with older age resembling morphology in humans (Archer et al. 2006). This model has two strengths: First it represents an experimental system that shows histologic characteristics similar to those from human disease. Secondly, it is based on pathophysiologic mechanisms that are known from hereditary chronic pancreatitis in humans. It therefore might be a model that most closely mimics the human pathophysiology. Moreover, R122H_mPRSS1 mice displayed an enhanced reaction upon serial caerulein injections: while

in wildtype animals the inflammatory reaction is largely resolved during the post-injection phase PRSS1 transgenic mice showed a chronic inflammatory response with extensive collagen deposition. Unfortunately, the findings have never been replicated in other laboratories and the model and the mouse are no longer available.

3.6.2 CFTR

Cystic fibrosis transmembrane conductance regulator (CFTR) is expressed on epithelial cells such as ductal cells and functions as a low conductance chloride ion selective channel (Wang et al. 2014). Its major function is believed to dilute and alkalinize the protein-rich acinar secretions, thereby preventing the formation of protein plugs (Chen and Ferec 2012). Various loss-of-function CFTR variants have been reported in patients with chronic pancreatitis, occurring in up to 37% in idiopathic and 15% in alcoholic chronic pancreatitis (Cohn et al. 1998; Sharer et al. 1998; Weiss et al. 2005).

Snouwaert and collaborators developed a murine model of cystic fibrosis by targeted disruption of the CFTR gene ($cftr^{m1UNC}$; UNC: University of North Carolina). Homozygous knockout mice displayed many features common to young human cystic fibrosis patients. Life expectancy of these mice was often no longer than 40 days resulting from intestinal obstruction causing death (Snouwaert et al. 1992). Although overt signs of chronic pancreatitis were absent in young mice pancreata of CFTR−/− mice showed at least mild features of early cystic fibrosis such as a dilation of the apical acinar lumen (Durie et al. 2004), impaired acinar endocytosis or acidification of the pancreatic juice (Freedman et al. 2001). In older animals (9–24 months) more severe changes were visible including a higher proportion of connective tissue areas, clogging of the pancreatic duct with mucus and concomitant duct dilation with loss of exocrine tissue, highly resembling human morphology (Durie et al. 2004; Dimagno et al. 2005). Untreated CFTR−/− mice showed a reduction of constitutive expression of pancreatic digestive proteins, lipase, pro-elastase and trypsinogen by around 20–25%. Upon caerulein hyperstimulation CFTR−/− mice developed more severe acute pancreatitis but displayed only a blunted increase of pancreatic digestive enzymes, which might suggest mild pancreatic exocrine insufficiency (Dimagno et al. 2005). Cell death was shifted from an apoptotic to a non-apoptotic form.

Limiting factors of this model imply the necessity of longer breeding periods to reach an older stage. In addition, other organs will be involved besides the pancreas because cystic fibrosis is a systemic disease affecting many organs. This means that this experimental model might not be an ideal system for studying pancreatic injury alone and its effects. Moreover life-threatening complications independent of pancreatic insufficiency can occur, such as respiratory airway obstruction due to defective mucociliary clearance or intestinal obstruction that cause early death of mice (Durie et al. 2004).

3.6.3 Cytokines/Chemokines

Many other genes are involved in acute and chronic inflammation and immune response in humans. Whether they affect human chronic pancreatitis as well is still a matter of debate and they will be good candidates for prospective genetic linkage analysis. During inflammation pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β) or TNFα and chemokines, e.g. CXCL1 and CXCL2 are released causing an influx of immune cells (Steele et al. 2015). Invading inflammatory cells themselves augment local damage, perpetuate protease activation and further release of cytokines (Sendler et al. 2013).

Transgenic mice with overexpression of human IL-1β (sshIL-1β) were followed up for 2 years. The pancreas showed typical histologic features of chronic pancreatitis, i.e. organ atrophy, dilatation of the pancreatic and biliary tract, secondary to proximal fibrotic stenosis (Marrache et al. 2008). Overt sign of exocrine or endocrine insufficiency were not observed in elastase sshIL-1β mice.

This model also addressed the question of malignant transformation in the setting of chronic inflammation. Pancreatic adenocarcinoma can arise on the basis of chronic pancreatitis and p53 mutations are frequently seen in pancreatic cancer (Hingorani et al. 2005; Tuveson and Hingorani 2005). When crossed with heterozygous $p53^{R172H/+}$ mice to create a double transgenic mouse an increased frequency of tubular complexes including some evidence of acinar-ductal metaplasia, that are considered to be preneoplastic lesions, were detected. However pancreatic ductal adenocarcinoma was hardly seen.

CXCR−/− mice were protected from pancreatic damage when chronic pancreatitis was induced by serial caerulein injections (Steele et al. 2015). In particular pancreata showed less organ atrophy and a reduced leukocyte infiltration. Although neutrophil infiltration significantly increases during acute and chronic pancreatitis selective neutrophil depletion (by anti-Ly6G antibody) was less effective suggesting that CXCR2 on non-neutrophils contributes to the development of chronic pancreatitis. Extension of fibrosis was comparable to wildtypes indicating that activation of stellate cells is still maintained despite CXCR2 knockout.

Monocyte chemoattractant protein 1 (MCP-1) is classified as a CC-chemokine. Rats were treated with dibutyltin dichloride (DBTC) and received intramuscular injections of mutant MCP-1 (mMCP-1) plasmids for several days. Pancreatic fibrosis induced by DBTC was attenuated by transgenic expression of mutant MCP-1. Concomitantly a decrease of serum MCP-1 concentrations and less inflammation were seen. Rats carrying mMCP-1 were heavier than controls suggesting that exocrine insufficiency is abrogated in these animals (Zhao et al. 2005).

3.6.4 Autoimmune Mediated

MRL/MpJ mice bearing a mutated lymphoproliferative gene, *lymphoproliferation* (*lpr*), (MRL/MpJ-lpr/lpr) spontenously develop autoimmune disorders such as glomerulonephritis, arthritis and sialadenitis. Mice lacking the *lpr*-gene also develop autoimmune diseases, but at later stage of life (Andrews et al. 1978; Kanno et al. 1992). Inflammatory lesions with acinar destruction and fatty tissue replacement were found in up to 74% of female mice at 34–38 weeks. Endocrine function was preserved, as pancreatic islets remained unaffected. Interestingly male mice later developed pancreatitis that was less intense and only present in less than 40% of 46–50 week old mice. Sex-related factors are discussed to cause the attenuated form, since administration of androgens retarded autoimmune disease in female mice as well (Steinberg et al. 1980). Histology of MRL/Mp mice, in particular their inflammatory infiltrate and fibrosis pattern was comparable with autoimmune pancreatitis type I as seen in humans. Characteristic signs including periductal lymphoplasmocytic infiltration, storiform fibrosis or elevated antibody levels (lactoferrin, carboanhydrase) were present (Schwaiger et al. 2014). Application of polyinosinic:polycytidylic acid (poly I:C) accelerated and enhanced disease progression. Blockage of cytotoxic T-lymphocyte associated protein 4 (CTLA-4), one of the most potent modulators of T-cell response, increased the severity of autoimmune pancreatitis as well (Schwaiger et al. 2014).

Lymphotoxin receptors are membrane proteins of the TNF superfamily and are involved in intracellular signaling. Pleiotropic functions including control of an adequate immune response are maintained lymphotoxins (Wolf et al. 2010). Mice with transgenic expression of lymphotoxin α and β (Ela1-LTαβ) resembled features of autoimmune pancreatitis. When lymphocytes were depleted (Ela1-LTab/Rag1(−/−)) autoimmunity was lost whereas deletion of monocytes (Ela1-LTab/Ccr2(−/−)) preserved autoimmune disease but prevented early pancreatic tissue damage (Seleznik et al. 2012).

C5 is a factor of the complement system and complement activation drives many inflammatory responses. Cleavage of the C5 molecule generates C5a and b. C5a exerts a predominant

pro-inflammatory activity mediating leukocyte chemotaxis and release of proinflammatory cytokines. An increased vascular permeability facilitates neutrophil transmigration (Kohl 2001). C5b holds cytolytic functions through the formation of the membrane attack complex (MAC) but also possess a multitude of non-cytolytic immune functions as well (Woodruff et al. 2011). Trypsin is known to act as a complement activator, and is able to cleave both C3 and C5 (Acioli et al. 1997).

C5 is functionally linked to liver fibrogenesis, as its receptor (C5R1) is expressed on endothelial and Kupffer cells and activates myofibroblasts. Deletion of C5, either genetically or pharmacologically resulted in reduced liver fibrosis upon CCL4 treatment (Hillebrandt et al. 2005). C5 exerts pro-fibrogenic effects in chronic pancreatitis as well. Sendler and coworkers subjected C5 deficient mice to either pancreatic duct ligation for up to 3 weeks or serial caerulein injections for 10 weeks. In both models pancreatic fibrosis was reduced in C5−/− animals and most predominant at later time points. Pharmacological anti-C5 treatment using a C5-receptor antagonist or a peptide inhibitor achieved comparable results to the knockout mouse model. Isolated pancreatic stellate cells were activated by C5a and synthesized massive amounts of extracellular proteins (Sendler et al. 2015).

3.6.5 WBN/Kob Rats

An example of a rat model mimicking chronic pancreatitis is given by the WBN/Kob (Wistar-Bonn/Kobori) strain. This strain is derived from Wistar rats and was originally generated as a model susceptible to gastric tumors. Starting with an age of 3–6 months male WBN/Kob rats show progressive fibrosis around the pancreatic ducts and vessels. A degradation of Langerhans islets leads to a reduction of number and size of the islets (Mori et al. 2009; Ohashi et al. 1990). At 9–12 months of age full manifestation of diabetes mellitus occurs with impaired glucose tolerance, hyperglycemia and glycosuria. Exocrine function measured by BT-PABA (n-benzoyl-l-tyrosyl-p-aminobenzoic acid) urinary excretion was diminished as well. Chromosomal mapping revealed two potentially responsible loci at chromosome 7 and X, Pdwk1 and 2 (pancreatitis and diabetes mellitus in WBN/Kob locus 1 and 2). Candidate genes were found in the Pdwk1 locus that makes a genetic origin of this phenotype likely (Mori et al. 2009).

More genetically engineered animals models exist describing chronic fibrotic disease in the pancreas. Good examples are a pancreas specific Kif3a knockout model that resulted in cyst formation and pancreatic fibrosis in aged mice. The Kif3a gene encodes a subunit of the kinesin-2 complex that is essential in cilia formation and its defect is associated with several human genetic diseases, including polycystic kidney disease (PCKD) Bardet-Biedl syndrome and primary ciliary dyskinesia (Cano et al. 2006). Perk−/− mice experienced a rapid loss of their endocrine and exocrine function accompanied with increasing cell death (Harding et al. 2001). Absence of PERK (Protein kinase R-line endoplasmic reticulum kinase), a transmembrane protein of the endoplasmic reticulum and usually highly expressed in the pancreas, renders cells to be more susceptible to ER stress and protein misfolding. For further information on genetic animal models for chronic pancreatitis please see Table 5.3.

Apparently the majority of studies using mouse models employ the C57BL/6 strain for their experiments. This strain is most frequently used in biomedical research. Moreover genetically modified mouse models are often derived from a C57BL/6 background. Meanwhile due to existence of various mouse breeding facilities and separate inbred colonies a high number of C57BL/6 substrains exist that have important genetic and phenotypic differences (Bourdi et al. 2011; Ulmasov et al. 2013; Watkins-Chow and Pavan 2008). In a recent study it could be shown that substrains of C57BL/6 mice show different disease severities of chronic pancreatitis

Table 5.3 Overview of genetic animal models for chronic pancreatitis (adapted from Lerch and Gorelick, 2013)

Gene	Function	Genetic manipulation (ko/tg)	Induction of CP	Morphology	Species	Author
Proteases/cellular components						
PRSS1 p.R122H	Cationic trypsinogen	tg (elastase promotor)	Spontaneous	Acinar cell necrosis, inflammatory cell infiltration, fibrosis, acinar dedifferentation	Mouse	Archer H, Gastroenterology 2006
CFTR	Chloride ion channel	ko	Spontaneous	Acinar cell atrophy, ductal dilatation, ductal clogging, fibrosis (at older age), no endocrine dysfunction	Mouse	Snouwaert JN, Science 1992
						Durie PR, Am J Pathol 2004
						DiMagno MJ, Gastroenterology 2005
KIf3a	Subunit of kinesin complex, cilia formation	ko (conditional)	Spontaneous	Fibrosis, acinar-ductal-metaplasia, lipomatosis, cysts	Mouse	Cano DA, Gastroenterology 2006
PERK	Prevents ER stress and protein misfolding	ko	Spontaneous	Cell death, exocrine and endocrine insufficiency	Mouse	Harding HP, Mol Cell 2001
Cytokines/chemokines						
Human IL-1β	Pro-inflammatory cytokine	tg (elastase promotor)	Spontaneous	Organ atrophy, dilatation of the pancreatic/biliary tract, no exocrine or endocrine insufficiency	Mouse	Marrache F, Gastroenterology 2008
Cxcr2	Chemokine receptor, neutrophil migration	ko	Caerulein	Less organ atrophy, less inflammation, no gross changes of fibrosis	Mouse	Steele CW, J Pathol 2015
MCP-1	Chemotaxis factor	tg (mutant MCP-1)	DBTC	Less fibrosis, less inflammation, less weight loss	Rat	Zhao HF, Gut 2005
C5	Complement factor, pro-inflammatory, chemotactic function	ko	Duct ligation, caerulein	Less fibrosis, reduced stellate cell activation, less collagen I synthesis	Mouse	Sendler M, Gastroenterology 2015

Autoimmune mediated						
MRL/MpJ	Lymphoproliferative gene	tg (mutant lpr)	Spontaneous	Signs of autoimmune pancreatitis, no endocrine insufficiency	Mouse	Kanno H, Clin Exp Immunol 1992 Andrews BS, J Exp Med 1978 Schwaiger T, Gut 2014
Lymphotoxin α and β	Intracellular signalling, immune response	Double tg (elastase promotor)	Spontaneous	Signs of autoimmune pancreatitis (type 2), no endocrine insufficiency	Mouse	Seleznik GM, Gastroenterology 2012
Other						
WBN/Kob	Pdwk1 and 2 (chromosome 7 and x), unknown function	Crossbreeding of Bonn (DE) and Kobori (Tokyo, JP) rats	Spontanous	Fibrosis, inflammatory infiltration, diabetes mellitus, reduced exocrine function	Rat	Ohashi K, Int J Pancreatol 1990 Mori M, Exp Anim 2009

ko knockout, *tg* transgenic, + yes, − no, *n.a.* no information available

upon repetitive caerulein injections. Fibrosis, acinar atrophy and inflammatory infiltrate were markedly more in B6J than in B6N substrains (Ulmasov et al. 2013). Knowledge of the exact background of genetically engineered animals and their control is of high importance, as an erroneous choice of the wrong genetic substrain most probably produces misleading results.

3.7 Concluding Remarks

Studies consistently showed that the available experimental models satisfactorily mimic histopathologic features of chronic pancreatitis whilst rodents (mice and rats) are investigated most thoroughly. There are different techniques that can be used to create pancreatic fibrosis and the most frequently used ones are ligation models and repetitive caerulein applications. Manifold combinations of techniques, often with additional dietary modifications (alcohol, fat) are practiced. There is increasing data on genetic animal models for chronic pancreatitis and some of them seem to imitate pathophysiology of humans quite well. Diabetes as the result of endocrine insufficiency was seen in only some models, however mostly after a long disease course and when the entire organ is affected.

In our opinion there is still a lack of usable models mimicking exocrine insufficiency. A small functioning part of the pancreas is sufficient to produce secretory digestive enzymes to avert clinically overt exocrine malfunction unless combined with a high fat diet. Probably exocrine function can only be impaired when using a model of very severe form of chronic pancreatitis. Secondly, other tools for measurement of exocrine function should be considered. In humans direct function tests are the gold standard that assess the secretion of enzymes and bicarbonate into the small intestine after stimulation. These enzymes are usually collected by an oro- or nasoduodenal tube and then quantitated. Definitely this procedure is invasive and time consuming and would be even more complicated in animals. Here further developments on indirect tests in animals will be necessary to overcome this problem.

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