Aus dem

CharitéCentrum 8 für Chirurgische Medizin

Chirurgische Klinik

Campus Charité Mitte | Campus Virchow-Klinikum

Direktor: Professor Dr. med. Johann Pratschke

Habilitationsschrift

Purinergic regulation of hepatic inflammation and fibrosis and implications for liver surgery

zur Erlangung der Lehrbefähigung

für das Fach Viszeralchirurgie

von

Dr. med. Linda Feldbrügge

Eingereicht: Dezember 2021

Dekan: Prof. Dr. Axel Radlach Pries

1. Gutachter: Prof. Dr. Clemens Schafmeyer, Rostock

2. Gutachterin: Prof. Dr. Christiane Bruns, Köln

CONTENT

ABBREVIATIONS

ADA	adenosine deaminase
ADP	adenosine monophosphate
AHR	aryl hydrocarbon receptor
ALT	alanine amino transferase
AMP	adenosine diphosphate
ASH	alcoholic steato-hepatitis
ATP	adenadenosine triphosphate
BBG	brilliant blue G
CCl4	carbon tetrachloride
CLP	cecal ligation and puncture
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
DAMP	danger associated molecular pattern
DDC	3,5-diethoxycarbonyl-1,4-dihydrocollidine
DSS	dextran-sulfate-sodium
ECM	extracellular matrix
ENTPD or NTPDase	ecto-nucleoside triphospho-diphosphohydrolase
HCC	hepatocellular carcinoma
HIF-1α	hypoxia inducible factor-1α
HSC	hepatic stellate cells
IL	interleukin
LPS	lipopolysaccharide
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steato-hepatitis
NF-kB	nuclear factor- kB
NKT cells	Natural killer T cells
PD-1	programmed cell death protein ligand 1
PDGF-β	platelet derived growth factor-β
PD-L1	programmed cell death protein 1
qPCR	quantitative polymerase chain reaction
ROS	reactive oxygen species
STAT3	signal transducer and activator of transcription3
TGF-β	transforming growth factor- β

1. INTRODUCTION

1.1. Clinical relevance of liver fibrosis

1.1.1. Overview: Liver inflammation, fibrosis and cirrhosis

The enormous capacity of the healthy liver to regenerate after injury, intoxication or partial resection, is famous [1]. However, in case of repeating or continuous insult to the liver tissue, healthy hepatocytes are replaced by excessively forming scar tissue, resulting in liver fibrosis. A central process of fibrogenesis is inflammation, initiated either directly by the trigger of liver injury, or secondarily by damaged and dying hepatocytes. Even then, the liver can regenerate and fibrotic tissue be partly resolved, if the cause is removed. If it persists, fibrosis may eventually progress to liver cirrhosis with destruction of normal tissue architecture and ultimately organ failure [2]. Etiologies for liver fibrosis most commonly include infectious, toxic and metabolic diseases, such as alcoholic and non-alcoholic steatohepatitis (ASH and NASH). Irrespective of the underlying liver disease, liver cirrhosis represents a pre-cancerous state for the development of hepatocellular carcinoma (HCC), which is the most common primary cancer from the liver, and the fifth most frequent cause of cancer related death in the world [3].

As of today, no specific anti-fibrotic treatment has shown convincing results in clinical trials, although many different mechanisms have been targeted and tried successfully in experimental studies [4]. Liver transplantation remains the only treatment option for end stage liver disease, if elimination of the trigger comes too late for fibrosis to resolve. Therefore, despite major insights into the pathophysiology of liver inflammation and liver fibrosis that research has yielded in the last years, further studies are needed to understand underlying mechanisms and search for anti-fibrotic treatment and prevention of HCC.

1.1.2. Considerations for the liver surgeon in the era of minimally invasive surgery

Surgical resection is the recommended treatment for resectable primary liver cancer (intrahepatic cholangiocarcinoma, iCC and HCC) or secondary malignancies, for example colorectal liver metastasis in non-cirrhotic livers [5, 6]. However, if HCC occurs in underlying liver cirrhosis, which is the case in about 80-90% [7], guidelines propose liver transplantation. First, this cures both the cancer and the underlying liver disease, and secondly, surgery has long been known to entail more perioperative risks for patients with liver cirrhosis [8]. Nevertheless, recent years show a tendency towards resection over transplantation due to increasing scarcity of donated organs on the one hand [9, 10], and reduced perioperative risks on the other hand. This latter development can be attributed in part to the advancement

of minimally invasive liver surgery [11, 12], which is considered to be especially beneficial for patients with liver cirrhosis [13]. One of the concerns for liver resection in cirrhosis is a higher risk for bleeding, caused, in short, by impaired coagulation and portal hypertension. A surgical strategy to control bleeding in liver resection, developed decades ago, is hepatic inflow occlusion during parenchymal resection, named after its describer the Pringle maneuver [14]. For as long as it has been practiced, there has been controversy about its potential deleterious effects on postoperative liver function, caused by ischemia-reperfusion injury [15, 16]. This has been proposed to be particularly relevant in patients with underlying liver cirrhosis [17]. In our center, minimally invasive techniques have, over the last seven years, become the predominant approach for liver resections [18, 19]. During this time, the indications for liver resection for HCC in cirrhosis have been slowly extended towards including more extensive resections and patients with more pronounced cirrhosis while complication rates remained comparable to non-cirrhotic patients [20].

As the limits are pushed, it is essential to continuously re-evaluate the safety and optimize technical strategies of surgery. Part of my research objective, parallel to conducting experimental studies on the pathogenesis of liver fibrosis, was therefore to determine the consequences of cirrhosis on perioperative risks in minimally invasive liver surgery.

1.2. Pathophysiology of hepatic and intestinal inflammation and fibrosis

1.2.1. Acute liver injury – the initial inflammatory stimulus

Causes for acute liver injury can be toxic, drug induced or infectious. If the trigger does not persist, leading to chronic injury and fibrosis, the organ usually recovers; however, in rare cases of excessive liver injury, it can lead to acute liver failure with high mortality [21]. The most common cause of acute liver failure in Western countries is acetaminophen (paracetamol) intoxication, which causes massive necrotic cell death through mitochondrial oxidative stress [22]. Irrespective of the underlying cause, massive necrotic cell death provokes the stimulation of innate immune cells. Most important players are Kupffer cells, the liver resident macrophages, that are activated by danger associated molecular patterns (DAMP), such as DNA fragments or ATP, released from necrotic hepatocytes [23]. This massive, and liver-specific process of sterile inflammation involves infiltration of neutrophils, blood derived monocytes, Natural Killer T cells (NKT cells) and other immune cells and has been shown to evoke further tissue injury by maintaining the secretion of pro-inflammatory signals, including reactive oxygen species (ROS) and cytokines. On the other hand, this immune response encompasses the initiation of immunoregulatory, pro-regenerative

processes like the differentiation of immunoregulatory macrophages and myofibroblasts, and is ultimately necessary for liver regeneration [24].

1.2.2. Continuous liver inflammation leads to liver fibrosis

In liver fibrosis, activated myofibroblasts produce excessive amounts of extracellular matrix (ECM), especially collagen. The main effector cells in this core process of fibrogenesis have been understood to be hepatic stellate cells (HSC) that are quiescent in the healthy liver, and can proliferate and transdifferentiate into myofibroblasts upon different stimuli [25]. The quantitative relevance of other cell types, including portal fibroblasts, that are also able to constitute myofibroblasts in the process of fibrogenesis, has been discussed controversially [26]. The multitude of pathways involved in HSC activation and its perpetuation are core targets for the development of anti-fibrotic strategies, and have been studied intensively with new mechanisms continually being discovered. HSC stimulation has been known to be caused by cytokines such as transforming growth factor-β (TGF-β) and platelet derived growth factor-β (PDGF-β), but also involves ROS, autophagy and ECM signaling to HSC [27]. Sterile liver inflammation is at the center of both the initiation and the progression of liver fibrosis, and a variety of immune cells can regulate myofibroblast activity and differentiation. As in the context of acute liver injury, macrophages are key players, involved in the activation of myofibroblasts and thus the initiation of fibrosis through secretion of TGF-β and other pro-fibrotic substances, but they are also required for fibrosis resolution [28, 29]. A simple, dichotomous model of macrophage polarization to a pro-inflammatory, classically activated M1 and an alternatively activated, immunoregulatory M2 macrophage has been abandoned with the realization of a much more complex, heterogeneous spectrum with a high plasticity [30]. To understand macrophages as key regulators of liver inflammation and fibrosis and discover ways of modulating them, it is crucial to consider the specific, highly inconsistent local microenvironment.

1.2.3. Inflammatory bowel disease

Considerable evidence links liver inflammation to intestinal inflammation. This “gut-liver axis” has been ascribed to interactions between the intestinal microbiome, immune cells and the liver, and it has been demonstrated to be a relevant mechanism contributing to alcoholic and non-alcoholic liver disease, HCC and other liver diseases [31-34]. Most research has focused on inflammatory bowel disease, which is present in 80% of patients with primary sclerosing cholangitis [35]. Inflammatory bowel disease, most commonly Crohn’s disease or ulcerative colitis, has deleterious consequences for patients’ quality of life, and was found to

increase mortality by up to 10% for ulcerative colitis and 50% for Crohn's disease compared to the general population [36]. The core pathophysiology of inflammatory bowel disease lies in aberrant innate and adoptive immune responses, caused by a still insufficiently understood interaction of genetic and environmental factors [37]. Treatment is aimed at suppressing immune response, but remains inefficient in many cases for unclear reasons [38].

One of the many pathways that have been found to contribute to perpetuated inflammation in inflammatory bowel disease, as well as in acute and chronic inflammation in the liver, is purinergic signaling. The exploration of this pathway in inflammatory and fibrotic diseases of the gut and liver provides some insights about the interface of metabolic and immune homeostasis and offers new potential targets for therapy.

1.3. Purinergic signaling

1.3.1. Ecto-nucleotidases in the purinergic signaling pathway

The system of purinergic signaling is made up of extracellular nucleosides and nucleotides as signaling molecules and their specific ionotropic or metabotropic purinergic receptors. Best described transmitters are adenosine triphosphate (ATP) and its derivates adenosine diphosphate (ADP) and adenosine [39]. ATP, which is present intracellularly in high concentrations, is released into the extracellular space in the event of cell damage or activation. The local extracellular ATP concentration can rise up to 30-fold after release through vesicular exocytosis or cell membrane channels [40]. It is a known DAMP that can initiate a strong pro-inflammatory immune response via P2X7 receptor mediated activation of inflammasome [41]. However, after secretion to the extracellular space, ATP is degraded quickly by cell surface-located ecto-enzymes: ATP is de-phosphorylated to ADP and to adenosine monophosphate (AMP) by ecto-nucleoside triphosphate diphosphohydrolases (E-NTPDases, ENTPD) and AMP further hydrolyzed by the ubiquitously expressed 5'nucleotidase (5'NT or CD73) to form adenosine, as depicted schematically in **Figure 1**.

ATP degradation products, ADP and adenosine can bind to their own specific receptors and exert different and partly opposing effects to those of ATP: adenosine is known to have immunoregulatory, pro-regenerative effects [42], which can be interpreted as a mechanism to automatically limit and resolve the local stress-induced, inflammatory reaction triggered by ATP. Thus, the extracellular concentrations of ATP and adenosine in a given local microenvironment decide the net effect of local purinergic signaling towards more pro-inflammatory or anti-inflammatory responses. ENTPDs, in concert with 5'NT, modulate the local extracellular space towards an immunoregulatory microenvironment by reducing ATP and increasing adenosine concentrations.

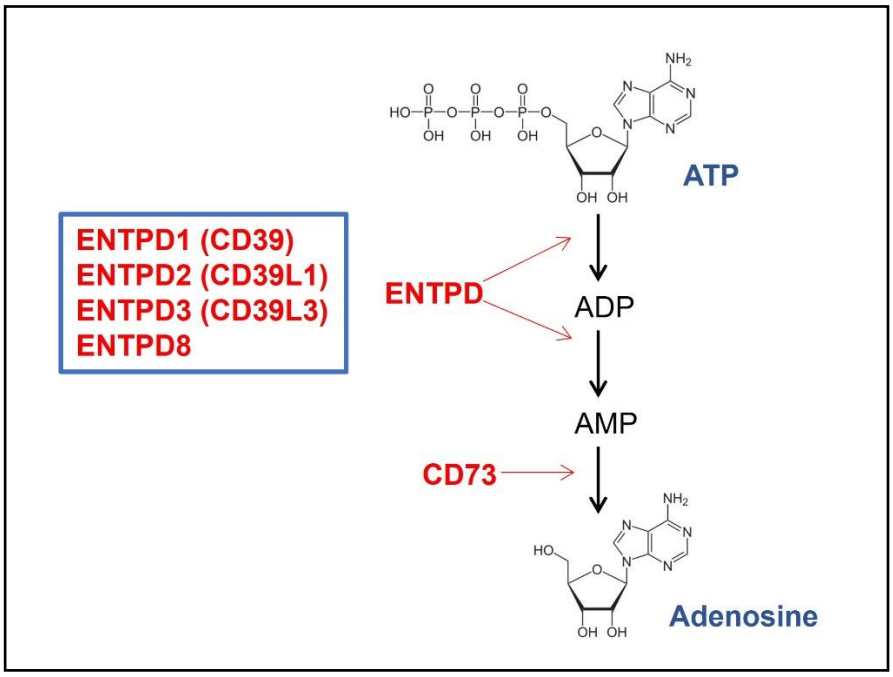

Figure 1. Degradation of extracellular adenosine triphosphate (ATP) to ultimately form adenosine, catalyzed by cell-surface enzymes ecto-nucleoside triphospho-diphosphohydrolase (ENDPD)-1, also known as CD39, ENTPD2 (CD39L1), ENTPD3 (CD39L3) and ENTPD8, and by CD73 (5'nucleotidase, 5NT).

1.3.2. CD39 and its role in immune regulation

Eight enzymes have been classified as ENTPDs, four of which are localized on the membrane surface and thus capable of modulating extracellular nucleotide and nucleoside concentrations, ENTPD1, ENTPD2, ENTPD3 and ENTPD8 [43]. ENTPD1 or CD39 is the best described ENTPD, and has been implicated in immunoregulatory effects of many cell types such as endothelial cells, vascular smooth muscle cells, B and T lymphocytes, macrophages and other myeloid derived immune cells. In macrophages, CD39 modulates autocrine purinergic signaling by scavenging extracellular ATP, producing adenosine and thus boosting transformation into an immunoregulatory phenotype [44]. ATP and adenosine signaling is transmitted by P2X7 and A2A and A2B receptors, respectively. ATP binding to P2X7 receptors leads to inflammasome activation within macrophages and subsequently to release of interleukin(IL)-1β and IL-18 [45]. Adenosine, generated via hydrolyzation of ATP by CD39 and 5'NT, drives macrophages to a more immunoregulatory phenotype through interaction with A2A and A2B receptors [46]. CD39 expression on macrophages is therefore essential for limiting inflammation in local or systemic inflammatory response, for example in sepsis. Consistent with this concept, CD39 deficient mice display a higher mortality from experimental sepsis than wild type mice [44, 47].

CD39 has also been displayed as an important steering element of immunoregulatory T cell populations. Early publications described the expression of CD39 on widely known regulatory T cell populations, such as CD4+CD25+ and Foxp3+ T cells and linked A2A and P2 signaling, modulated by CD39, to their immunosuppressive functions [48, 49]. Over the last ten years, several studies defined a variety of further subgroups of CD4+ T cells that express CD39 and described their roles in human and experimental disease: Foxp3- type 1 regulatory (Tr1) cells [50], γδ+ T cells [51] and T helper 17 (Th17) cells [52]. Recently, CD39 expression on CD8+ T cells was investigated, where it seems to represent a marker of exhaustion [53], and has also been implicated in anti-tumor cytotoxic T cell activity [54]. Several mechanisms have been described that regulate CD39 expression and through this regulatory T cell function, including metabolic signals such as hypoxia inducible factor-1α (HIF-1α), bilirubin and aryl hydrocarbon receptor (AHR) [50, 55]. Of note, CD39 expression has also been shown on other cells of the immune system, including B cells, granulocytes, dendritic cells and others [56], where it has also been reported to regulate inflammation and immunity, but which are not the focus of the presented research.

1.3.3. Non-CD39 ENTPDs

While numerous studies have focused on CD39 and its diverse roles in inflammation, immunity and other processes, far less is known about the other related ecto-nucleotidases. ENTPD8 is widely expressed on the surface of hepatocytes and secretory cells of exocrine organs [57], where its functions have not been elucidated and no specific immune-regulatory role has yet been reported. ENTPD8 will not be a subject of the presented research. The other two ENTPDs of the CD39 family are ENTPD2 (initially called CD39-like-enzyme 1, CD39L1) and ENTPD3 (CD39L3). While the protein structure of the three ecto-nucleotidases (ENTPD1, 2 and 3) is similar, the expression pattern on different cells and tissues shows striking differences: CD39 is predominantly expressed on immune cells as well as endothelial cells. ENTPD2 on the other hand, is found on perivascular cells, periportal fibroblasts in the liver, and on both nerve cells and glial cells in the nervous system [58, 59]. No direct association of ENTPD2 with immune cells has been described. Based on its localization in periportal areas and *in vitro* studies, ENTPD2 was found to regulate the proliferation of bile duct cells and presumed to play a role in biliary fibrosis [60]. Apart from that, it is expressed by neural progenitor cells and regulates progenitor cell proliferation [59].

Similar to ENTPD2, ENTPD3 is expressed on cells of the nervous system, but unlike both CD39 and ENTPD2, it was also described on exocrine and endocrine tissues, with a particularly strong expression on pancreatic islet cells [61]. It was further found on the surface of hepatic stellate cells, specifically in their activated state [62], suggesting a

potential role in the pathophysiology of liver fibrosis. Interestingly, one study explored the expression of non-CD39-ENTPDs on macrophages and found ENTPD3 expression on alternatively activated M2 macrophages [63]. Of note, the three ENTPDs also differ in their enzyme kinetics: CD39 has a high affinity to ADP, resulting in almost no noticeable ADP accumulation in the process of ATP degradation, but instead immediate generation of AMP. In contrast, ENTPD2 is a preferential ATPase and produces locally high ADP concentrations with slower conversion to AMP, and further to adenosine. ENTPD3 is between the other two enzymes with slower conversion of ATP and some intermediate ADP production [43].

1.4. Ecto-nucleotidases in hepatic and intestinal inflammation

As depicted in the last chapters, purinergic signaling is ubiquitously present and modulates inflammation and immunity. ENTPD, that serve as a “switch” to limit inflammation, have been found to play a relevant role in both hepatic and intestinal inflammation in both experimental models and clinical disease. Most research has so far been conducted on CD39, while far less is known about the role of other ENTPD.

In acute liver injury, provoked by ischemia and reperfusion, CD39 has been shown to be protective from exacerbated inflammation and cell death in animal models [64, 65]. The same was seen in a mouse model of acetaminophen induced acute liver injury, where mice deficient for CD39 displayed significantly more liver necrosis, more hepatic hemorrhage and an enhanced vascular injury [66, 67]. Liver regeneration after acute injury, such as partial hepatectomy, has also been demonstrated to be facilitated through CD39 [68]. The importance of other ENTPD in acute liver injury is unclear.

The roles of purinergic signaling in liver fibrosis are complex: ATP signaling via both P2Y and P2X receptors has been shown to promote fibrosis through activation of hepatic stellate cells [69-71]. Interestingly, adenosine signaling through A2A receptor, expressed on hepatic stellate cells, has also been reported to exert pro-fibrotic effects [72]. This is in keeping with findings on a protective effect of caffeine in liver fibrosis, as caffeine is a non-selective adenosine receptor antagonist [73]. The effects of CD39 as a regulator of both ATP and adenosine concentrations are therefore not easy to predict in this scenario. ENTPD2 has been studied in descriptive studies of its hepatic and intestinal expression pattern and in *in vitro* studies, where a potential role for regulation of bile duct proliferation was suggested [58, 60]. However, neither ENTPD2 nor ENTPD3 have been studied in animal models of liver fibrosis or human studies.

CD39 expression was found to protect mice from experimental intestinal inflammation induced by dextran-sulfate-sodium (DSS) treatment [74]. These effects could be attributed to

immunoregulatory modulations of T cells. Several mechanisms of regulation of CD39 expression on relevant, T regulatory and Th17 T cell populations have since been discovered [55, 75]. Importantly, this regulatory role of CD39 in colitis was shown to be relevant both in animal experiments as well as human disease [52, 76]. So far, other ENTPD have not been studied in the context of intestinal inflammation.

1.5. Objective

The goal of this research project was to further elucidate the roles of ecto-nucleotidases of the CD39 family, especially CD39-like enzymes, ENTPD2 and ENTPD3, in the inflammation and fibrosis of the gut and the liver. A further objective was to demonstrate the clinical relevance of liver cirrhosis for liver surgery, using as an example the effect of hepatic inflow occlusion in minimally invasive hepatic resection.

2. PRESENTATION OF OWN WORK

2.1. Safety of hepatic inflow occlusion for liver resection in cirrhotic patients

Ortiz Galindo, S.A.; Haber, P.K.; Benzing, C.; Krenzien, F.; Riddermann, A.; Frisch, O.; Schöning, W.; Schmelzle M., Pratschke J.; **Feldbrügge, L.** Safety of intermittent Pringle maneuver during minimally invasive liver resection in patients with hepatocellular carcinoma with and without cirrhosis. *Langenbeck's Archive of Surgery.* Nov. 2021. https://doi.org/10.1007/s00423-021-02361-z.

Minimally invasive liver surgery has been increasingly used for HCC resection over the last years. It has been convincingly shown to reduce perioperative risks in retrospective studies and meta-analyses. Some evidence suggests that patients with liver cirrhosis benefit especially. However, little is known about the use of the Pringle maneuver in this group of patients, as cirrhotic livers might be more susceptible to ischemia-reperfusion injury. While it may be a useful tool in these patients with their tendency to bleed, some studies have reported an increased risk for perioperative liver failure.

To evaluate the safety of minimally invasive Pringle maneuver in patients with HCC, we retrospectively compared perioperative outcome parameters between cases with, and those without use of the Pringle maneuver. A propensity score matching was performed to control for a potential selection bias with regards to surgical complexity. A subgroup analysis focused on patients with liver cirrhosis.

No increase in perioperative complication rates was observed in patients who underwent hepatic inflow occlusion during minimally invasive HCC resection, taking into account surgical difficulty and presence of liver cirrhosis. Laboratory tests for liver function were also similar between groups. Duration of surgery seemed longer in cases that included use of the Pringle maneuver, but this difference was only visible before propensity score matching, making it likely to be confounded by surgical difficulty. Intraoperative transfusion rate, used as a surrogate marker for blood loss, showed no difference between groups.

In summary, using the minimally invasive Pringle maneuver in patients with HCC, including those who have underlying liver cirrhosis, does not seem to cause clinically relevant ischemia-reperfusion injury, and can be safely applied if deemed useful [77].

The results of this study add another piece to understanding the clinical implications of liver fibrosis and cirrhosis for liver surgery. The following chapter focuses on its pathophysiology and discusses purinergic signaling and ecto-nucleotidases as one of the underlying pathways that regulate liver inflammation, fibrosis and cirrhosis. Gaining insight into these mechanisms is the basis for finding new therapeutic targets to prevent liver fibrosis and HCC (refer to 2.2).

2.2. Roles of ecto-nucleotidases in liver inflammation and fibrosis

2.2.1. Macrophage-expressed CD39 is protective in biliary fibrosis

Rothweiler, S; **Feldbrügge, L**; Jiang, ZG; Csizmadia, E; Longhi, MS; Vaid, K; Enjyoji, K; Popov, YV; Robson, SC. Selective deletion of ENTPD1/CD39 in macrophages exacerbates biliary fibrosis in a mouse model of sclerosing cholangitis. *Purinergic Signalling* 2019, 15, 375-385. https://doi.org/10.1007/s11302-019-09664-3.

In previous work, we observed more fibrosis in mice globally deficient for CD39. In this study, we aimed to determine if macrophages are the main responsible cells for this phenotype. To this end, we generated myeloid-specific *Cd39* knockout mice and subjected them to DDC feeding to induce biliary type fibrosis. In comparison, we used wild type and global *Cd39* knockout mice.

We first confirmed the specific deletion of CD39 on myeloid cells by qPCR, flow cytometry and immunohistochemistry. After induction of liver fibrosis by DDC, both globally *Cd39* null mice as well as myeloid-specific *Cd39* knockout mice displayed more severe fibrosis when compared to their respective wild type controls, as assessed by hepatic collagen content and upregulation of profibrogenic factors, α-smooth muscle actin (α-SMA) and transforming growth factor-β (TGF-β). While these findings certainly suggest a regulatory role of macrophage-expressed CD39 in the induction of biliary type fibrosis, we did not find any significant differences in commonly used markers of classically (M1) or alternatively (M2) activated macrophages between globally or myeloid-specific *Cd39* deficient mice and wild type controls.

Taken together, our findings provide more evidence for the protective function of CD39 in liver inflammation and fibrosis, and indicate a role of myeloid cells, most likely liver residing macrophages. However, the exact mechanism of regulation of fibrosis remains to be elucidated. A simple bipolar model of macrophage polarization with CD39 promoting an anti-inflammatory phenotype could not be demonstrated [78].

Unlike CD39, ENTPD2 is expressed on portal fibroblasts that have been shown to form myofibroblasts in liver fibrosis, especially biliary type fibrosis. We therefore aimed to study its role in experimental fibrosis using knockout mice (refer to 2.2.2).

2.2.2. Distinct roles of NTPDase2 in liver regeneration and fibrosis

Feldbrügge, L.; Jiang, Z.G.; Csizmadia, E.; Mitsuhashi, S.; Tran, S.; Yee, E.U.; Rothweiler, S.; Vaid, K.A.; Sévigny, J.; Schmelzle, M.; et al. Distinct roles of ecto-nucleoside triphosphate diphosphohydrolase-2 (NTPDase2) in liver regeneration and fibrosis. *Purinergic Signalling* 2018, 14, 37-46. https://doi.org/10.1007/s11302-017-9590-3.

ENTPD2 has been found exclusively in the functional compartment of portal areas, and has been proposed to regulate the proliferation of bile duct cells by reducing extracellular ATP. Deletion of CD39 in mice enhances the severity of liver fibrosis in experimental models for fibrosis, as was previously shown (refer to 2.2.1). The aim of this study was to investigate whether ENTPD2 also has protective functions in liver fibrogenesis, and whether this is limited to biliary fibrosis.

To answer these questions we induced liver fibrosis in wild type mice and *Entpd2* null mice in two different established rodent models of liver fibrosis: by administration of carbon tetrachloride (CCl4) or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC). We further performed partial hepatectomy to study a potential role of ENTPD2 catalyzed purine degradation on liver cell regeneration.

Total ENTPD2 expression in wild type livers is increased as assessed by qPCR after induction of fibrosis as well as following partial hepatectomy. Immunohistochemistry, including immunofluorescent double staining shows that ENTPD2 expressing portal fibroblasts spread to fibrotic areas in CCl4 induced fibrosis, while expression patterns were unaltered in the DDC model. Interestingly, *Entpd2* null mice were affected more severely by fibrosis induction through CCl4, displaying significantly more production of collagen. In contrast, no difference was seen after DDC feeding. Both liver regeneration (liver weights) and hepatocyte proliferation (Ki67 positive cells) were similar between wild type and *Entpd2* null mice after partial hepatectomy, suggesting a negligible role of ENTPD2 in the regulation of normal hepatocyte regeneration.

Our results suggest an inducible protective role of ENTPD2 in liver fibrosis caused by CCl4, that may be synergistic with previously observed effects by CD39 [79].

While CCl4 causes liver necrosis as a trigger for fibrosis induction, the most commonly used, and clinically relevant, model for acute (necrotizing) liver injury is acetaminophen intoxication. This model was used for the study presented next, investigating the role of ENTPD2 in acute liver injury (refer to 2.2.3).

2.2.3. ENTPD2 alleviates acetaminophen-induced hepatotoxicity

Feldbrügge, L.*; Splith, K.*; Kämmerer, I.; Richter, S.; Riddermann, A.; Ortiz Galindo, S.A.; Krenzien, F.; Müller, T.; Csizmadia, E.; Pratschke, J.; Robson, S.C.; Schmelzle, M. Ecto-Nucleotide Triphosphate Diphosphohydrolase-2 (NTPDase2) Deletion Increases Acetaminophen-Induced Hepatotoxicity. *International Journal of Molecular Sciences* Aug 20 2020, 21 https://doi.org/10.3390/ijms21175998

Acetaminophen intoxication is one of the most common cause for acute liver failure. It has therefore been widely used for an experimental model of acute liver injury in rodents. In the liver, CD39 is expressed on endothelial cells and Kupffer cells, and has been shown to alleviate acute liver injury induced by acetaminophen intoxication by scavenging the danger molecule ATP. Hepatic expression of CD39-like enzyme ENTPD2 is less abundant than CD39, and mostly limited to periportal fibroblasts within portal areas.

The aim of this experimental study was to investigate a potential role that ENTPD2 may play in modulating the local hepatic micro-milieu in acute liver injury. To this end, we induced acute liver injury by acetaminophen injection in globally *Entdp2* null mice and wild type mice and assessed the degree of liver injury by quantifying histological areas of necrosis and serum levels of alanine amino transferase (ALT). Commonly used markers for intrahepatic inflammation, regeneration and fibrogenesis were then analyzed by quantitative polymerase chain reaction (qPCR) and immunohistochemistry.

We observed significantly enhanced acute liver injury in *Entpd2* null mice after acetaminophen intoxication when compared to wild type mice, as measured by necrotic area and serum ALT. In wild type mice, ENTPD2 was upregulated after induction of acute liver injury, without changing its expression pattern. Inflammatory cytokines IL-6 and PDGF-β were elevated significantly earlier and (in the case of IL-6) more prolonged in *Entpd2* null mice. No differences were found in hepatocyte proliferation as assessed by immunohistochemistry for Ki67 or in the hepatic expression levels of fibrogenesis-related proteins, vimentin, desmin, collagen or α-smooth-muscle-actin.

Taken together, we here show for the first time that ENTPD2, similarly to CD39, has protective effects in acute liver injury induced by acetaminophen, despite its limited and specific hepatic expression in the portal areas [80].

As CD39-dependent immunoregulatory mechanisms have been described in both hepatic and intestinal inflammation, contributing potentially to the above-mentioned “gut-liver axis”, the aim of the following study was to explore the role of ENTPD2 and ENTPD3 in inflammatory bowel disease (refer to 2.3).

2.3. Roles of ecto-nucleotidases in intestinal inflammation

Feldbrügge, L.; Moss, A.C.; Yee, E.U.; Csizmadia, E.; Mitsuhashi, S.; Longhi, M.S.; Sandhu, B.; Stephan, H.; Wu, Y.; Cheifetz, A.S.; et al. Expression of Ecto-nucleoside Triphosphate Diphosphohydrolases-2 and -3 in the Enteric Nervous System Affects Inflammation in Experimental Colitis and Crohn's Disease. *Journal of Crohns & Colitis* 2017, 11, 1113-1123. https://doi.org/10.1093/ecco-jcc/jjx058.

The association of CD39 with regulation of (auto-)immunity and inflammation of the intestine has been demonstrated in numerous studies and has been ascribed mainly to the expression on different T cell populations. In the case of CD39-like enzymes, ENTPD2 and ENTPD3, this association with immune regulation is less obviously understood, as these enzymes are not directly found on immune cells in relevant amounts. However, as our studies have indicated a similar function in immune regulation for ENTPD2 as for CD39 in inflammatory liver disease (2.2.2 and 2.2.3), we aimed to explore the roles of ENTPD2 and 3 in intestinal inflammation, as both enzymes are abundantly expressed in the enteric nervous system, which was shown to be implicated with inflammatory processes in the gut.

To this end, we induced acute colitis by feeding DSS in wild type mice and global knockout mice for ENTPD2, and ENTPD3, respectively. We observed more severe colitis in both knockout mice when compared to wild type, most prominently in *Entpd2* null mice. We further observed a more pronounced pro-inflammatory polarization of colonic macrophages isolated from *Entpd2* null colons. Immunohistochemistry showed the known expression pattern of both enzymes in cells of the enteric nervous system, especially glial cells, and no relevant expression on infiltrating immune cells.

To test a potential clinical relevance of our findings, we measured ADPase activity in the blood of patients with different stages of Crohn’s disease and compared them with healthy controls. Here, we could show that total ADPase activity was significantly reduced in Crohn’s patients when compared with controls. This ADPase activity seemed to be inhibitable by a chemical blocking agent to ENTPD2 and -3 and correlated with disease severity.

In summary, ENTPD2 and ENTPD3 are both expressed in human and murine enteric nervous system and are involved in the immune regulation of acute colitis, particularly ENTPD2 [81].

3. DISCUSSION

This work summarizes the findings of four experimental studies on the role of purinergic signaling, modulated by ecto-enzymes, in the immune regulation of hepatic and intestinal inflammation [78-81], and includes an exemplary investigation of the clinical consequences for the liver surgeon [77].

The liver has always been a challenge but also a fascination for surgeons, due to its high risk of bleeding on one, and its high capacity for regeneration on the other hand [82]. When liver resection had gradually been turned into a standard procedure with acceptable mortality and morbidity, liver fibrosis and cirrhosis were still understood to present a limit [83]. In recent years, advances in surgical techniques and perioperative medicine have pushed these limits further and rendered the presence of liver cirrhosis a relative contraindication to liver resection at most [84]. One important development was the rise of minimally invasive liver surgery. In several retrospective and prospective observational studies accompanying the development of our own center for minimally invasive liver surgery – first laparoscopic, then robotic – we demonstrated the safety of these new approaches [19, 85-88]. Other centers have recently published randomized-controlled trials showing superiority of minimally invasive over open techniques [89, 90]. With the transition of open to minimally invasive surgery, surgical techniques and devices have been transferred from open surgery and continuously modified for optimal use in laparoscopy [91-93]. While every center that performs minimally invasive liver surgery constantly tests variations to improve outcomes and efficiency, more reproducible and available evidence on particular technical aspects is needed to further refine state of the art laparoscopic liver resection. In **publication 1** [77], both laparoscopic and robotic liver resections for HCC were analyzed with a focus on the safety of minimally invasive intermittent Pringle maneuver. As the majority of HCC patients are diagnosed with liver cirrhosis, this group of patients can be considered at increased risk for perioperative complications [94]. With regard to the use of the Pringle maneuver, cirrhotic patients have always been discussed controversially: on the one hand they have a higher risk for bleeding during parenchymal resection, on the other hand, they may be more likely to suffer perioperative impairment of liver function [17, 95]. In our cohort, we observed no higher occurrence of adverse outcomes in cirrhotic patients that underwent Pringle maneuver during minimally invasive liver resection, after propensity score matching to control for selection bias. It must be noted, that this study does not prove superiority of using Pringle maneuver with regards to reduction of blood loss nor was it designed to answer that question. However, these data suggest that its use does not increase the risk even for cirrhotic patients, and may be used at the discretion of the surgeon if it aids a safe and controlled operation.

While it is necessary for the liver surgeon to constantly and critically review surgical techniques and innovations to improve treatment of patients with liver cirrhosis, understanding the underlying pathophysiology is at least of equal importance. Only the knowledge of causes and regulatory mechanisms of liver inflammation and fibrogenesis will advance treatment and prevention of cirrhosis and HCC. The role of purinergic signaling as one of these regulatory pathways is investigated in **publications 2-5** and will be discussed in the following section.

Inflammation is the organism's response to any stimulus that is perceived as harmful. An intact inflammatory response is vital to survive infectious diseases and prevent the development of cancer. On the other hand, the resolution of inflammation and initiation of wound healing and tissue regeneration is just as important, demonstrated by auto-immune diseases as cases of excessive, uncontrolled inflammation. To govern the balance between initiation and resolution of inflammation, a complex system of interacting pathways has evolved that controls the activation, differentiation and proliferation of immune cells. The discovery of many of these pathways and their targeting to develop anti-inflammatory drugs has revealed a vast redundancy and interdependence of these pathways, leading to inexplicable treatment failures, for example in inflammatory bowel disease [96, 97]. A better understanding of the intricate modulation of inflammation and immunity will help refine treatment strategies, for example by targeting complementary signaling pathways in combination therapy.

One of these signaling pathways regulating inflammation and immunity is the system of purinergic signaling. CD39 and related enzymes constitute an immunoregulatory "switch" by abrogating the danger signal ATP and producing regulatory adenosine [98]. Over the last years, this mechanism has been elucidated in numerous studies on autoimmune diseases, both clinical and experimental, including multiple sclerosis [99, 100], allergic asthma [101], rheumatoid arthritis [102], diabetes [103] and inflammatory bowel disease [52, 104]. Some manipulations of CD39 expression and activity have already been proposed as potential treatment for these hyper-inflammatory diseases [55, 75, 105, 106]. However, using these insights for treating inflammatory diseases is not as straightforward as it may seem. Our recent studies on CD39 and other ecto-enzymes revealed several important aspects that should be considered with regards to a potential CD39-based anti-inflammatory treatment and will be discussed below.

In a previous study [107], we investigated acute liver injury in sepsis and found that liver injury was in large parts mediated by ATP signaling via P2X7. It could be prevented completely by P2X7 inhibition in wild type mice, but not in mice lacking CD39. This exemplifies the dual role of CD39 in modulating purinergic signaling with ATP reduction on one, and adenosine production on the other hand. Accordingly, the effect of P2X7 inhibition

in wild type mice could be reached in *Cd39* null mice after combination with A2A receptor blockade. Macrophages have previously been shown to control their own activation status by secreting high amounts of ATP, which could be expected to stimulate inflammasome activation through P2X7 activation, but instead, through rapid hydrolyzation by CD39, led to an adenosine transmitted immunoregulatory response [44]. It was already known that the effects of CD39 deletion (or inhibition) can be paradoxical through P2 receptor desensitization by increased local concentration of ATP [108, 109]. These feedback and autocrine regulatory mechanisms underline the high plasticity of CD39 and purinergic receptor functions and their dependence on the local micro-milieu. Consequently, therapeutic intervention into CD39 activity may yield unpredicted results.

Earlier studies had already displayed a higher susceptibility of global *Cd39* null mice to liver inflammation and fibrosis in a genetic and a toxic model of sclerosing cholangitis and biliary fibrosis [110]. This was mainly linked to an increased hepatic infiltration of CD8+ T cells from the gut, while the role of macrophages was not investigated. In **publication 2** [78], we therefore used the same toxic model of cholangitis and fibrosis in mice with a selective, macrophage-specific deletion of CD39. We could show that these mice, like global knockout mice, exhibited enhanced inflammation and fibrosis, pointing to a relevant involvement of CD39+ liver macrophages. Interestingly, we did not observe expression of typical macrophage markers of immune regulation, which may imply a distinct polarization that cannot be classified into the simplified model of pro- versus anti-inflammatory macrophage types. Altered ATP and adenosine signaling may also be more important in other cells, such as HSC, in this model, than in macrophages themselves. Of note, a recent publication investigated the role of CD39 in a model of alcoholic liver fibrosis and found that pharmacological CD39 blockade with POM1 *alleviated* liver injury and fibrosis [111], which is in contrast with our own findings. Both studies are difficult to compare as they investigate pathophysiologically distinct diseases, where CD39 might in fact have different effects. Importantly, pharmacological inhibition of CD39 with POM1 has lower specificity and efficacy than genetic deletion or antibody blockade [112]. However, as both ATP and adenosine have been shown to convey pro-fibrotic signals to HSC, it is conceivable that CD39 blockade can have different effects in different disease models, depending the type of involved immune cells and myofibroblasts and their state of differentiation.

Taken together, our studies confirm the important role CD39 plays in modulating immune response in liver inflammation and fibrosis. While CD39 clearly contributes to dampening excessive inflammation in acute liver injury in sepsis [107], the effects in chronic inflammation and liver fibrosis are more complex due to partly synergistic effects of ATP (decreased by CD39) and adenosine (increased by CD39) [78].

Another very important aspect in the evaluation of CD39 based therapy for inflammatory disease is the flipside to CD39 mediated immunosuppression: its role in cancer development and progression. With the emergence and the success of immune checkpoint inhibitors such as antibodies to cytotoxic T-lymphocyte-associated protein (CTLA)-4, programmed cell death protein (PD)-1 or PD-L1 as anti-cancer treatment [113, 114], the striking potential of boosting the antitumor immune response became apparent. In earlier years of research on CD39, a few studies had investigated the role of CD39 in tumor immunology and found that genetic deletion or pharmacologic inhibition could protect mice against tumor development or tumor spread [115-117]. However, in recent years, along with the triumphant story of checkpoint inhibition, many more studies were published, several already including preclinical testing of CD39 blockade, alone or in combination with systemic chemotherapy or already established immune checkpoint inhibitors [112, 118]. These studies are interesting, as systemic pharmacological inhibition of CD39, in contrast to what might be expected from our studies on global *Cd39* knockout mice, does not seem to be deleterious, judging from available reports on adverse events [119]. In light of these recent developments, an immune therapy *boosting* CD39 activity to achieve immune regulation in autoimmune disease has to be evaluated carefully for the risk of carcinogenic effects.

In contrast to CD39, other ecto-nucleotidases, namely ENTPD2 and ENTPD3, have not been described on the surface of immune cells in meaningful amounts [43]. While ENTPD3 expression is reportedly mostly limited to nervous, exocrine and endocrine tissues [61, 120, 121], ENTPD2 is expressed in liver tissue and has been proposed to regulate bile duct proliferation via local ATP degradation [58, 60]. ENTPD2 can therefore be presumed to cause similar alterations in local extracellular nucleotide and nucleoside homeostasis in the liver as CD39, but with diverse physiological consequences due to their distinct cellular distribution. We conducted several experimental studies to investigate the specific role of ENTPD2 in liver inflammation and fibrosis, using *Entpd2* null mice. In **publications 3** [79] and **4** [80], we report the results from four different experimental models of liver disease, including partial hepatectomy, acetaminophen intoxication, CCl4 treatment and DDC feeding. We observed that in all cases of liver injury, the expression of ENTPD2 is upregulated in the liver in wild type mice. Functionally, ENTPD2 expression seemed to play a role in acute (induced by acetaminophen) and chronic necrotic liver injury (induced by long-term CCl4 administration), where it had protective properties, while the enzyme appeared inessential for proliferation induction after partial hepatectomy or immune regulation in the DDC model of biliary inflammation and fibrosis [79, 80].

ENTPD2 expression in the liver is limited to portal areas [58, 79] and is generally less abundant in the healthy liver parenchyma than CD39 [57]. It is therefore plausible that it does not play a major role in the regulation of hepatocyte proliferation after a standard model of

partial hepatectomy, a situation where ductular reaction contributes only marginally to proliferation [122], because the enzyme is not expressed in close enough proximity to the proliferating cells. Liver injury that involves large amounts of tissue necrosis, however, as produced by both acetaminophen and CCl4, seems to require some regulation by ENTPD2, illustrated by the aggravated phenotype in *Entpd2* null mice in both models. In the case of CCl4 induced liver fibrosis, we observed a change in the distribution of ENTPD2 expressing cells with spreading into the liver parenchyma. As these cells maintained double staining with a portal fibroblast marker, this may correspond to a proliferation and expansion of portal fibroblasts that could contribute to the pool of myofibroblasts. Of note, this hypothesis can be discussed controversially: Some evidence points towards the main source of myofibroblasts in liver fibrosis to be HSC [25, 123], while other studies also present portal fibroblasts as potential contributors [26, 124]. Interestingly, in the DDC model of biliary inflammation and fibrosis, no significant increase in fibrosis was observed in *Entpd2* null mice (unlike *Cd39* null mice, [78]), contrary to what had been expected due to the distinct expression in portal areas. This may be due to the considerable infiltration of immune cells that carry CD39 and may overpower the effect of ENTPD2 deletion on the relatively small portion of portal fibroblasts. In fact, overexpression of one ENTPD to compensate the genetic deletion of another has been described before and may generally limit the interpretation of genetic knockout models for the study of individual ENTPDs [125].

Interestingly, a recent publication displays a novel aspect on ENTPD2 in the liver: Chiu et al. observed that hypoxia induces ENTPD2 expression in hepatocellular carcinoma (HCC) via HIF-1α signaling and that ENTPD2 mediated ATP degradation enhances myeloid derived suppressor cell function and promotes tumor growth [126]. As HIF-1α is known to be induced also in liver fibrosis [127, 128], this might be another explanation for the observed upregulation of ENTPD2 and the relative protection from fibrosis displayed at least in the CCl4-induced model [79]. Since the article by Chiu et al. on the role of ENTPD2 in HCC was published, a small number of highly interesting studies were added very recently that seem to confirm the pro-tumor effect of ENTPD2 in different cancer types: both Chen et al. and Cui et al. included ENTPD2 as one of five and six genes, respectively, that were associated with a worse outcome in HCC [129, 130]. Wu et al. developed an “immune prognostic model”, based on ENTPD2 and one other gene that identified high-risk cases in lung adenocarcinoma [131].

Our findings point towards a protective role of ENTPD2 in liver inflammation and fibrosis, similarly to CD39, especially in toxic necrotic disease models. As discussed for the case of CD39 before, a potential pharmacological manipulation of ENTPD2 activity has to be viewed critically, in light of current studies on their involvement in cancer tolerance.

CD39 has been known to be protective, not only in sterile liver inflammation, but also in inflammatory bowel disease. This could be demonstrated both in human as well as in experimental murine colitis [74, 76]. The anti-inflammatory effect was mostly attributed to its expression on different subsets of infiltrating T cells (T regulatory cells, Th17 cells and γδ+ T cells) [52, 74]. Intriguingly, as demonstrated in **publication 5** [81], mice also suffer from more severe acute experimental colitis in case of global deletion of ENTPD2 or ENTPD3, although neither of the enzymes is found on immune cells.

Both ecto-nucleotidases have been known to be present in the nervous system [43]. ENTPD2 is expressed in taste bud cells, neural progenitor cells, in the hippocampus and in glial cells of the peripheral nervous system [59, 132-134]. ENTPD3 is also found in the central and peripheral nervous system, for example in nociceptors [121, 135]. The roles of both enzymes within the nervous system have not been fully explained. Some of the initially presumed functions, based on their expression patterns and enzyme kinetics, were actually later questioned, such as the role of ENTPD2 in eye formation [136], or the regulation of nociception in mice [137]. Furthermore, global knockout mice for both enzymes have been available to researchers for several years, and no study has yet been published that reports a major disruption of neurological functions in these mice.

With regards to the enteric nervous system, neither ecto-nucleotidase had previously been studied in detail. In our study, the expression patterns of both enzymes in the nerve and glial cells of the intestinal wall were similar with a broader expansion of ENTPD2 positive cells into the submucosa and lamina propria [81]. We subjected both *Entpd2* and *Entpd3* null mice to DSS to induce colitis. *Entpd2* null mice displayed significantly more severe colitis and *Entpd3* null mice significantly more intestinal bleeding, when compared to wild type mice. Intestinal wall macrophages had a more pro-inflammatory phenotype in *Entpd2* null mice. Other studies had revealed the interplay between the enteric nervous system and immune cells in the pathogenesis of inflammatory bowel disease [138]. We interpreted our findings as ENTPD2-mediated polarization of enteric macrophages towards a regulatory phenotype, which would explain its protective role in colitis. The stronger phenotype of ENTPD2 compared to ENTPD3 may be explained by the slight differences in localization, with ENTPD2 expression more abundant in immune-cell-rich areas closer to the mucosa. Furthermore, a recent study could establish a direct link between glial cells and enteric macrophages in experimental colitis [139]. The same authors corroborated our own findings of ENTPD2 mediated protection from experimental colitis and could additionally show a gut barrier dysfunction in *Entpd2* null mice with colitis as well as a lower density of myenteric neurons [140]. The latter may be due to ENTPD2 mediated control of neural proliferation [141].

To test a potential translation of our findings from animal models to human disease, we also examined ENTPD2 and ENTPD3 expression in human intestinal tissues, both healthy and inflamed, and saw a very similar constellation to mice. We further performed enzyme activity tests in the plasma of patients with Crohn's disease and could demonstrate elevated plasma ADPase activity, most of which was sensitive to POM6, an inhibitor of ENTPD2 and ENTPD3. Both POM1 (CD39 associated) and POM6-sensitive ADPase activity were lower with higher Crohn's disease activity [81]. Although we cannot prove which cells or particles are the origin of this blood-borne ADPase activity, we have previously shown that cellular microparticles are abundant in human plasma and carry ecto-nucleotidases with quantifiable enzyme activity [142], suggesting an increased shedding of ENTPD-positive microparticles from destroyed intestinal wall layers in inflammatory bowel disease.

4. SUMMARY

Liver cirrhosis, known to increase intra- and postoperative complications after hepatic resection, has always been considered a limiting factor by the liver surgeon. Minimally invasive surgery, including laparoscopic and robotic techniques, is becoming the standard approach and seems to benefit cirrhotic patients especially. We analyzed the effect of hepatic inflow occlusion in minimally invasive liver resection in patients with HCC and cirrhosis, and found no increase in complications or liver failure. While clinical studies like these need to accompany the development of new surgical techniques to re-evaluate risks for individual patient groups, and keep cirrhotic patients from being excluded from optimal treatments, it is also crucial to better understand the underlying mechanisms regulating inflammation, fibrosis and cirrhosis.

Acute inflammation of the gut or the liver, caused by toxic, infectious or metabolic triggers, is usually self-limited and leads to *restitutio ad integrum*. If the inflammatory stimulus persists, chronic inflammation can ensue. In the gut, chronic inflammatory bowel disease is characterized by recurrent episodes of enteritis and increased risk of cancer. In the liver, chronic inflammation, as seen in alcoholic or non-alcoholic fatty liver disease, viral hepatitis or auto-immune hepatitis, leads to liver fibrosis, and eventually liver cirrhosis with a risk for organ failure and cancer. Inflammation and its resolution are finely regulated by a multitude of interconnected signaling pathways, including purinergic signaling. Signal transmitters are extracellular nucleotides and nucleosides, such as ATP, a pro-inflammatory "danger signal" and adenosine, an immunoregulatory and pro-regenerative stimulus. The balance between ATP and its derivate adenosine is regulated by a group of cell-surface localized enzymes, ecto-nucleotide triphosphate diphosphohydrolases (ENTPD). CD39 (ENTPD1) is located on

immune cells and leads to their immunoregulatory differentiation. ENTPD2 and 3 are similar enzymes that are expressed on portal fibroblasts and pericytes, and on endocrine and exocrine tissues, respectively. Both enzymes are also strongly expressed in nerve and glial cells.

In the presented research project, different experimental models of acute and chronic, intestinal and hepatic inflammation were performed using mice with global or targeted deficiency for CD39, ENTPD2 or ENTPD3. These studies confirmed the central role that these ecto-enzymes play as a “switch” turning a local micro-milieu into an immunoregulatory environment. Macrophage-expressed CD39 was found to alleviate sclerosing cholangitis and biliary fibrosis. ENTPD2, while not affecting liver regeneration after partial hepatectomy, attenuates acute necrotic liver injury and liver fibrosis. ENTPD2 and, to a lesser extent ENTPD3 have protective properties in experimental colitis, most likely due to their expression on the enteric nervous system.

Taken together, these findings expose the crucial role that CD39 and other ENTPD play through modulating purinergic signaling in creating an immunoregulatory microenvironment in gut and liver. These insights contribute to a better understanding of inflammation and anti-inflammatory medication in autoimmune diseases of liver and intestine.

5. REFERENCES

1. Fausto N, Campbell JS, Riehle KJ (2006) Liver regeneration. Hepatology 43:S45-S53
2. Hernandez-Gea V, Friedman SL (2011) Pathogenesis of Liver Fibrosis. Annual Review of Pathology: Mechanisms of Disease 6:425–456
3. Global Burden of Disease Liver Cancer C, Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, Al-Raddadi R, Alvis-Guzman N, Amoako Y, Artaman A, Ayele TA, Barac A, Bensenor I, Berhane A, Bhutta Z, Castillo-Rivas J, Chitheer A, Choi JY, Cowie B, Dandona L, Dandona R, Dey S, Dicker D, Phuc H, Ekwueme DU, Zaki MS, Fischer F, Furst T, Hancock J, Hay SI, Hotez P, Jee SH, Kasaeian A, Khader Y, Khang YH, Kumar A, Kutz M, Larson H, Lopez A, Lunevicius R, Malekzadeh R, McAlinden C, Meier T, Mendoza W, Mokdad A, Moradi-Lakeh M, Nagel G, Nguyen Q, Nguyen G, Ogbo F, Patton G, Pereira DM, Pourmalek F, Qorbani M, Radfar A, Roshandel G, Salomon JA, Sanabria J, Sartorius B, Satpathy M, Sawhney M, Sepanlou S, Shackelford K, Shore H, Sun J, Mengistu DT, Topor-Madry R, Tran B, Ukwaja KN, Vlassov V, Vollset SE, Vos T, Wakayo T, Weiderpass E, Werdecker A, Yonemoto N, Younis M, Yu C, Zaidi Z, Zhu L, Murray CJL, Naghavi M, Fitzmaurice C (2017) The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. JAMA Oncol 3:1683-1691
4. Roehlen N, Crouchet E, Baumert TF (2020) Liver Fibrosis: Mechanistic Concepts and Therapeutic Perspectives. Cells 9:875
5. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft DK, AWMF) (2019) S3-Leitlinie Kolorektales Karzinom, Kurzversion 2.1, 2019, AWMF Registrierungsnummer: 021/007OL.
6. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft DK, AWMF) (2021) Diagnostik und Therapie des Hepatozellulären Karzinoms und biliärer Karzinome, Langversion 2.0, 2021, AWMF Registernummer: 032/-053OL.
7. Forner A, Llovet JM, Bruix J (2012) Hepatocellular carcinoma. Lancet 379:1245-1255
8. Hackl C, Schlitt HJ, Renner P, Lang SA (2016) Liver surgery in cirrhosis and portal hypertension. World J Gastroenterol 22:2725-2735
9. DSO Jahresbericht 2010. Available online (accessed on 05.02.2021): https://www.klinikum-stuttgart.de/fileadmin/user_upload/dso_jb2010_d_www.pdf.
10. DSO Jahresbericht 2019. Available online (accessed on 05.02.2021): https://dso.de/SiteCollectionDocuments/DSO-Jahresbericht%202019.pdf.
11. Xourafas D, Pawlik TM, Cloyd JM (2019) Early Morbidity and Mortality after Minimally Invasive Liver Resection for Hepatocellular Carcinoma: a Propensity-Score Matched Comparison with Open Resection. J Gastrointest Surg 23:1435-1442
12. Ruzzenente A, Bagante F, Ratti F, Alaimo L, Marques HP, Silva S, Soubrane O, Endo I, Sahara K, Beal EW, Lam V, Poultsides GA, Makris EA, Popescu I, Alexandrescu S, Martel G, Workneh A, Hugh TJ, Guglielmi A, Aldrighetti L, Pawlik TM (2020) Minimally Invasive Versus Open Liver Resection for Hepatocellular Carcinoma in the Setting of Portal Vein Hypertension: Results of an International Multi-institutional Analysis. Ann Surg Oncol 27:3360-3371
13. Coletta D, De Padua C, Parrino C, De Peppo V, Oddi A, Frigieri C, Grazi GL (2020) Laparoscopic Liver Surgery: What Are the Advantages in Patients with Cirrhosis and Portal Hypertension? Systematic Review and Meta-Analysis with Personal Experience. J Laparoendosc Adv Surg Tech A 30:1054-1065
14. Pringle JH (1908) V. Notes on the Arrest of Hepatic Hemorrhage Due to Trauma. Ann Surg 48:541-549
15. Iwasaki J, Afify M, Bleilevens C, Klinge U, Weiskirchen R, Steitz J, Vogt M, Yagi S, Nagai K, Uemoto S, Tolba RH (2019) The Impact of a Nitric Oxide Synthase Inhibitor (L-NAME) on Ischemia(-)Reperfusion Injury of Cholestatic Livers by Pringle Maneuver and Liver Resection after Bile Duct Ligation in Rats. Int J Mol Sci 20:2114

16. Capussotti L, Muratore A, Ferrero A, Massucco P, Ribero D, Polastri R (2006) Randomized clinical trial of liver resection with and without hepatic pedicle clamping. Br J Surg 93:685-689
17. Sugiyama Y, Ishizaki Y, Imamura H, Sugo H, Yoshimoto J, Kawasaki S (2010) Effects of intermittent Pringle's manoeuvre on cirrhotic compared with normal liver. Br J Surg 97:1062-1069
18. Schmelzle M, Wabitsch S, Haber PK, Krenzien F, Kästner A, Biebl M, Öllinger R, Pratschke J (2019) Laparoskopische Leberchirurgie – Berliner Zentrumserfahrungen aus 250 konsekutiven Fällen. Zentralblatt fur Chirurgie 144:145–152
19. Feldbrügge L, Ortiz Galindo SA, Frisch O, Benzing C, Krenzien F, Riddermann A, Kästner A, Nevermann NF, Malinka T, Schöning W, Pratschke J, Schmelzle M (2021) Safety and feasibility of robotic liver resection after previous abdominal surgeries. Surg Endosc. Epub ahead of print (doi: 10.1007/s00464-021-08572-1)
20. Haber PK, Wabitsch S, Krenzien F, Benzing C, Andreou A, Schöning W, Öllinger R, Pratschke J, Schmelzle M (2019) Laparoscopic liver surgery in cirrhosis - Addressing lesions in posterosuperior segments. Surgical oncology 28:140–144
21. Bernal W, Lee WM, Wendon J, Larsen FS, Williams R (2015) Acute liver failure: A curable disease by 2024? Journal of Hepatology 62:S112-120
22. Ramachandran A, Jaeschke H (2019) Acetaminophen Hepatotoxicity. Semin Liver Dis 39:221-234
23. Woolbright BL, Jaeschke H (2017) Role of the inflammasome in acetaminophen-induced liver injury and acute liver failure. J Hepatol 66:836-848
24. Kubes P, Jenne C (2018) Immune Responses in the Liver. Annu Rev Immunol 36:247-277
25. Mederacke I, Hsu CC, Troeger JS, Huebener P, Mu X, Dapito DH, Pradere J-P, Schwabe RF (2013) Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. Nature Communications 4:2823
26. Lua I, Li Y, Zagory JA, Wang KS, French SW, Sévigny J, Asahina K (2016) Characterization of hepatic stellate cells, portal fibroblasts, and mesothelial cells in normal and fibrotic livers. J Hepatol 64:1137-1146
27. Tsuchida T, Friedman SL (2017) Mechanisms of hepatic stellate cell activation. Nature Reviews Gastroenterology & Hepatology 14:397-411
28. Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, Wu S, Lang R, Iredale JP (2005) Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest 115:56-65
29. Seki E, Schwabe RF (2015) Hepatic inflammation and fibrosis: functional links and key pathways. Hepatology (Baltimore, Md) 61:1066–1079
30. Krenkel O, Tacke F (2017) Liver macrophages in tissue homeostasis and disease. Nat Rev Immunol 17:306-321
31. Szabo G (2015) Gut-liver axis in alcoholic liver disease. Gastroenterology 148:30-36
32. Ponziani FR, Bhoori S, Castelli C, Putignani L, Rivoltini L, Del Chierico F, Sanguinetti M, Morelli D, Paroni Sterbini F, Petito V, Reddel S, Calvani R, Camisaschi C, Picca A, Tuccitto A, Gasbarrini A, Pompili M, Mazzaferro V (2019) Hepatocellular Carcinoma Is Associated With Gut Microbiota Profile and Inflammation in Nonalcoholic Fatty Liver Disease. Hepatology 69:107-120
33. Sun J, Zhang J, Wang X, Ji F, Ronco C, Tian J, Yin Y (2020) Gut-liver crosstalk in sepsis-induced liver injury. Crit Care 24:614
34. Tilg H, Zmora N, Adolph TE, Elinav E (2020) The intestinal microbiota fuelling metabolic inflammation. Nat Rev Immunol 20:40-54
35. Trapecar M, Communal C, Velazquez J, Maass CA, Huang YJ, Schneider K, Wright CW, Butty V, Eng G, Yilmaz O, Trumper D, Griffith LG (2020) Gut-Liver Physiomimetics Reveal Paradoxical Modulation of IBD-Related Inflammation by Short-Chain Fatty Acids. Cell Syst 10:223-239.e229
36. Jess T, Frisch M, Simonsen J (2013) Trends in overall and cause-specific mortality among patients with inflammatory bowel disease from 1982 to 2010. Clin Gastroenterol Hepatol 11:43-48

37. Guan Q (2019) A Comprehensive Review and Update on the Pathogenesis of Inflammatory Bowel Disease. J Immunol Res 2019:7247238
38. Volk N, Siegel CA (2019) Defining Failure of Medical Therapy for Inflammatory Bowel Disease. Inflamm Bowel Dis 25:74-77
39. Burnstock G, Campbell G, Bennett M, Holman M (1963) Inhibition of the smooth muscle on the taenia coli. Nature 200:581–582
40. Feranchak AP, Fitz JG, Roman RM (2000) Volume-sensitive purinergic signaling in human hepatocytes. Journal of Hepatology 33:174–182
41. Gombault A, Baron L, Couillin I (2012) ATP release and purinergic signaling in NLRP3 inflammasome activation. Frontiers in immunology 3:414
42. Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, Chen J-F, Enjyoji K, Linden J, Oukka M, Kuchroo VK, Strom TB, Robson SC (2007) Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. Journal of Experimental Medicine 204:1257–1265
43. Zimmermann H, Zebisch M, Sträter N (2012) Cellular function and molecular structure of ecto-nucleotidases. Purinergic Signal 8:437–502
44. Cohen HB, Briggs KT, Marino JP, Ravid K, Robson SC, Mosser DM (2013) TLR stimulation initiates a CD39-based autoregulatory mechanism that limits macrophage inflammatory responses. Blood 122:1935–1945
45. Franceschini A, Capece M, Chiozzi P, Falzoni S, Sanz JM, Sarti AC, Bonora M, Pinton P, Di Virgilio F (2015) The P2X7 receptor directly interacts with the NLRP3 inflammasome scaffold protein. The FASEB Journal 29:2450–2461
46. Csoka B, Selmeczy Z, Koscso B, Nemeth ZH, Pacher P, Murray PJ, Kepka-Lenhart D, Morris SM, Gause WC, Leibovich SJ, Hasko G (2012) Adenosine promotes alternative macrophage activation via A2A and A2B receptors. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 26:376–386
47. Csóka B, Németh ZH, Törő G, Koscsó B, Kókai E, Robson SC, Enjyoji K, Rolandelli RH, Erdélyi K, Pacher P, Haskó G (2015) CD39 improves survival in microbial sepsis by attenuating systemic inflammation. The FASEB Journal 29:25–36
48. Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, Chen JF, Enjyoji K, Linden J, Oukka M, Kuchroo VK, Strom TB, Robson SC (2007) Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med 204:1257-1265
49. Borsellino G, Kleinewietfeld M, Di Mitri D, Sternjak A, Diamantini A, Giometto R, Hopner S, Centonze D, Bernardi G, Dell'Acqua ML, Rossini PM, Battistini L, Rotzschke O, Falk K (2007) Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood 110:1225–1232
50. Mascanfroni ID, Takenaka MC, Yeste A, Patel B, Wu Y, Kenison JE, Siddiqui S, Basso AS, Otterbein LE, Pardoll DM, Pan F, Priel A, Clish CB, Robson SC, Quintana FJ (2015) Metabolic control of type 1 regulatory T cell differentiation by AHR and HIF1-α. Nature medicine 21:638–646
51. Libera J, Wittner M, Kantowski M, Woost R, Eberhard JM, de Heer J, Reher D, Huber S, Haag F, Schulze Zur Wiesch J (2020) Decreased Frequency of Intestinal CD39(+) γδ(+) T Cells With Tissue-Resident Memory Phenotype in Inflammatory Bowel Disease. Front Immunol 11:567472
52. Longhi MS, Moss A, Bai A, Wu Y, Huang H, Cheifetz A, Quintana FJ, Robson SC, Hogan SP (2014) Characterization of Human CD39+ Th17 Cells with Suppressor Activity and Modulation in Inflammatory Bowel Disease. PLoS One 9:e87956
53. Gupta PK, Godec J, Wolski D, Adland E, Yates K, Pauken KE, Cosgrove C, Ledderose C, Junger WG, Robson SC, Wherry EJ, Alter G, Goulder PJ, Klenerman P, Sharpe AH, Lauer GM, Haining WN (2015) CD39 Expression Identifies Terminally Exhausted CD8+ T Cells. PLoS Pathog 11:e1005177
54. Zhu W, Zhao Z, Feng B, Yu W, Li J, Guo H, Yang R (2021) CD8+CD39+ T Cells Mediate Anti-Tumor Cytotoxicity in Bladder Cancer. Onco Targets Ther 14:2149-2161

55. Longhi MS, Vuerich M, Kalbasi A, Kenison JE, Yeste A, Csizmadia E, Vaughn B, Feldbrugge L, Mitsuhashi S, Wegiel B, Otterbein L, Moss A, Quintana FJ, Robson SC (2017) Bilirubin suppresses Th17 immunity in colitis by upregulating CD39. JCI insight 2
56. Allard D, Allard B, Stagg J (2020) On the mechanism of anti-CD39 immune checkpoint therapy. J Immunother Cancer 8:e000186
57. Fausther M, Lecka J, Kukulski F, Lévesque SA, Pelletier J, Zimmermann H, Dranoff JA, Sévigny J (2007) Cloning, purification, and identification of the liver canalicular ecto-ATPase as NTPDase8. American journal of physiology Gastrointestinal and liver physiology 292:G785-795
58. Dranoff JA, Kruglov EA, Robson SC, Braun N, Zimmermann H, Sévigny J (2002) The ecto-nucleoside triphosphate diphosphohydrolase NTPDase2/CD39L1 is expressed in a novel functional compartment within the liver. Hepatology (Baltimore, Md) 36:1135–1144
59. Gampe K, Stefani J, Hammer K, Brendel P, Pötzsch A, Enikolopov G, Enjyoji K, Acker-Palmer A, Robson SC, Zimmermann H (2014) NTPDase2 and Purinergic Signaling Control Progenitor Cell Proliferation in Neurogenic Niches of the Adult Mouse Brain. Stem Cells 33:253-264
60. Jhandier MN, Kruglov EA, Lavoie EG, Sévigny J, Dranoff JA (2005) Portal fibroblasts regulate the proliferation of bile duct epithelia via expression of NTPDase2. The Journal of biological chemistry 280:22986–22992
61. Lavoie EG, Gulbransen BD, Martín-Satué M, Aliagas E, Sharkey KA, Sévigny J (2011) Ectonucleotidases in the digestive system: focus on NTPDase3 localization. American journal of physiology Gastrointestinal and liver physiology 300:G608-620
62. Andrade CMB, Wink MR, Margis R, Borojevic R, Battastini AMO, Guma FCR (2010) Changes in E-NTPDase 3 expression and extracellular nucleotide hydrolysis during the myofibroblast/lipocyte differentiation. Molecular and cellular biochemistry 339:79–87
63. Zanin RF, Braganhol E, Bergamin LS, Campesato LFI, Filho AZ, Moreira JCF, Morrone FB, Sevigny J, Schetinger MRC, de Souza Wyse AT, Battastini AMO (2012) Differential macrophage activation alters the expression profile of NTPDase and ecto-5'-nucleotidase. PLoS One 7:e31205
64. Hart ML, Gorzolla IC, Schittenhelm J, Robson SC, Eltzschig HK (2010) SP1-Dependent Induction of CD39 Facilitates Hepatic Ischemic Preconditioning. The Journal of Immunology 184:4017–4024
65. Yoshida O, Kimura S, Jackson EK, Robson SC, Geller DA, Murase N, Thomson AW (2013) CD39 expression by hepatic myeloid dendritic cells attenuates inflammation in liver transplant ischemia-reperfusion injury in mice. Hepatology 58:2163–2175
66. Schmelzle M, Splith K, Andersen LW, Kornek M, Schuppan D, Jones-Bamman C, Nowak M, Toxavidis V, Salhanick SD, Han L, Am Schulte Esch J, Jonas S, Donnino MW, Robson SC (2013) Increased plasma levels of microparticles expressing CD39 and CD133 in acute liver injury. Transplantation 95:63–69
67. Hoque R, Sohail MA, Salhanick S, Malik AF, Ghani A, Robson SC, Mehal WZ (2012) P2X7 receptor-mediated purinergic signaling promotes liver injury in acetaminophen hepatotoxicity in mice. Am J Physiol Gastrointest Liver Physiol 302:G1171-1179
68. Beldi G, Wu Y, Sun X, Imai M, Enjyoji K, Csizmadia E, Candinas D, Erb L, Robson SC (2008) Regulated Catalysis of Extracellular Nucleotides by Vascular CD39/ENTPD1 Is Required for Liver Regeneration. Gastroenterology 135:1751–1760
69. Dranoff JA, Ogawa M, Kruglov EA, Gaça MDA, Sévigny J, Robson SC, Wells RG (2004) Expression of P2Y nucleotide receptors and ectonucleotidases in quiescent and activated rat hepatic stellate cells. American journal of physiology Gastrointestinal and liver physiology 287:G417-424
70. Dranoff JA, Kruglov EA, Abreu-Lanfranco O, Nguyen T, Arora G, Aurora G, Jain D (2007) Prevention of liver fibrosis by the purinoceptor antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate (PPADS). In vivo (Athens, Greece) 21:957–965
71. Wu X, Wang Y, Wang S, Xu R, Lv X (2017) Purinergic P2X7 receptor mediates acetaldehyde-induced hepatic stellate cells activation via PKC-dependent GSK3beta pathway. International Immunopharmacology 43:164–171

72. Chiang DJ, Roychowdhury S, Bush K, McMullen MR, Pisano S, Niese K, Olman MA, Pritchard MT, Nagy LE (2013) Adenosine 2A receptor antagonist prevented and reversed liver fibrosis in a mouse model of ethanol-exacerbated liver fibrosis. PLoS One 8:e69114
73. Liu F, Wang X, Wu G, Chen L, Hu P, Ren H, Hu H (2015) Coffee Consumption Decreases Risks for Hepatic Fibrosis and Cirrhosis: A Meta-Analysis. PLoS One 10:e0142457
74. Gibson DJ, Elliott L, McDermott E, Tosetto M, Keegan D, Byrne K, Martin ST, Rispens T, Cullen G, Mulcahy HE, Cheifetz AS, Moss AC, Robson SC, Doherty GA, Ryan EJ (2015) Heightened Expression of CD39 by Regulatory T Lymphocytes Is Associated with Therapeutic Remission in Inflammatory Bowel Disease. Inflamm Bowel Dis 21:2806-2814
75. Robles RJ, Mukherjee S, Vuerich M, Xie A, Harshe R, Cowan PJ, Csizmadia E, Wu Y, Moss AC, Chen R, Robson SC, Longhi MS (2020) Modulation of CD39 and Exogenous APT102 Correct Immune Dysfunction in Experimental Colitis and Crohn's Disease. J Crohns Colitis 14:818-830
76. Friedman DJ, Künzli BM, A-Rahim YI, Sevigny J, Berberat PO, Enjyoji K, Csizmadia E, Friess H, Robson SC (2009) From the Cover: CD39 deletion exacerbates experimental murine colitis and human polymorphisms increase susceptibility to inflammatory bowel disease. Proceedings of the National Academy of Sciences of the United States of America 106:16788–16793
77. Ortiz Galindo SA, Haber PK, Benzing C, Krenzien F, Riddermann A, Frisch O, Schöning W, Schmelzle M, Pratschke J, Feldbrügge L (2021) Safety of intermittent Pringle maneuver during minimally invasive liver resection in patients with hepatocellular carcinoma with and without cirrhosis. Langenbecks Arch Surg. Epub ahead of print (doi: 10.1007/s00423-021-02361-z)
78. Rothweiler S, Feldbrügge L, Jiang ZG, Csizmadia E, Longhi MS, Vaid K, Enjyoji K, Popov YV, Robson SC (2019) Selective deletion of ENTPD1/CD39 in macrophages exacerbates biliary fibrosis in a mouse model of sclerosing cholangitis. Purinergic Signal 15:375-385
79. Feldbrügge L, Jiang ZG, Csizmadia E, Mitsuhashi S, Tran S, Yee EU, Rothweiler S, Vaid KA, Sévigny J, Schmelzle M, Popov YV, Robson SC (2018) Distinct roles of ecto-nucleoside triphosphate diphosphohydrolase-2 (NTPDase2) in liver regeneration and fibrosis. Purinergic Signal 14:37-46
80. Feldbrügge L, Splith K, Kämmerer I, Richter S, Riddermann A, Ortiz Galindo SA, Krenzien F, Müller T, Csizmadia E, Pratschke J, Robson SC, Schmelzle M (2020) Ecto-Nucleotide Triphosphate Diphosphohydrolase-2 (NTPDase2) Deletion Increases Acetaminophen-Induced Hepatotoxicity. Int J Mol Sci 21
81. Feldbrügge L, Moss AC, Yee EU, Csizmadia E, Mitsuhashi S, Longhi MS, Sandhu B, Stephan H, Wu Y, Cheifetz AS, Müller CE, Sévigny J, Robson SC, Jiang ZG (2017) Expression of Ecto-nucleoside Triphosphate Diphosphohydrolases-2 and -3 in the Enteric Nervous System Affects Inflammation in Experimental Colitis and Crohn's Disease. J Crohns Colitis 11:1113-1123
82. Foster JH (1991) History of Liver Surgery. Arch Surg 126:381-387
83. Choi TK (1988) Surgical resection for hepatocellular carcinoma. Ann Acad Med Singap 17:96-100
84. Romano F, Chiarelli M, Garancini M, Scotti M, Zago M, Cioffi G, De Simone M, Cioffi U (2021) Rethinking the Barcelona clinic liver cancer guidelines: Intermediate stage and Child-Pugh B patients are suitable for surgery? World J Gastroenterol 27:2784-2794
85. Feldbrügge L, Langenscheidt A, Krenzien F, Schulz M, Krezdorn N, Kamali K, Hinz A, Bartels M, Fikatas P, Schmelzle M, Pratschke J, Benzing C (2021) Health-Related Quality of Life and Mental Health after Surgical Treatment of Hepatocellular Carcinoma in the Era of Minimal-Invasive Surgery: Resection versus Transplantation. Healthcare (Basel) 9
86. Feldbrügge L, Wabitsch S, Benzing C, Krenzien F, Kästner A, Haber PK, Atanasov G, Andreou A, Öllinger R, Pratschke J, Schmelzle M (2020) Safety and feasibility of

laparoscopic liver resection in patients with a history of abdominal surgeries. HPB (Oxford) 22:1191-1196
87. Haber PK, Maier C, Kästner A, Feldbrügge L, Ortiz Galindo SA, Geisel D, Fehrenbach U, Biebl M, Krenzien F, Benzing C, Schöning W, Pratschke J, Schmelzle M (2021) Predicting the Risk of Postoperative Complications in Patients Undergoing Minimally Invasive Resection of Primary Liver Tumors. J Clin Med 10
88. Wabitsch S, Kästner A, Haber PK, Feldbrügge L, Winklmann T, Werner S, Pratschke J, Schmelzle M (2019) Laparoscopic versus open hemihepatectomy-a cost analysis after propensity score matching. Langenbecks Arch Surg 404:469-475
89. Fretland ÅA, Dagenborg VJ, Bjørnelv GMW, Kazaryan AM, Kristiansen R, Fagerland MW, Hausken J, Tønnessen TI, Abildgaard A, Barkhatov L, Yaqub S, Røsok BI, Bjørnbeth BA, Andersen MH, Flatmark K, Aas E, Edwin B (2018) Laparoscopic Versus Open Resection for Colorectal Liver Metastases: The OSLO-COMET Randomized Controlled Trial. Annals of surgery 267:199–207
90. Robles-Campos R, Lopez-Lopez V, Brusadin R, Lopez-Conesa A, Gil-Vazquez PJ, Navarro-Barrios Á, Parrilla P (2019) Open versus minimally invasive liver surgery for colorectal liver metastases (LapOpHuva): A prospective randomized controlled trial. Surg Endosc 30:3926-3936
91. Haber PK, Wabitsch S, Krenzien F, Benzing C, Andreou A, Schoning W, Ollinger R, Pratschke J, Schmelzle M (2019) Laparoscopic liver surgery in cirrhosis - Addressing lesions in posterosuperior segments. Surg Oncol 28:140-144
92. Coelho FF, Kruger JA, Fonseca GM, Araujo RL, Jeismann VB, Perini MV, Lupinacci RM, Cecconello I, Herman P (2016) Laparoscopic liver resection: Experience based guidelines. World J Gastrointest Surg 8:5-26
93. Benzing C, Krenzien F, Atanasov G, Seehofer D, Sucher R, Zorron R, Pratschke J, Schmelzle M (2015) Single incision laparoscopic liver resection (SILL) - a systematic review. GMS Interdiscip Plast Reconstr Surg DGPW 4:Doc17
94. Tanabe G, Sakamoto M, Akazawa K, Kurita K, Hamanoue M, Ueno S, Kobayashi Y, Mitue S, Ogura Y, Yoshidome N, et al. (1995) Intraoperative risk factors associated with hepatic resection. Br J Surg 82:1262-1265
95. Fagenson AM, Gleeson EM, Nabi F, Lau KN, Pitt HA (2021) When does a Pringle Maneuver cause harm? HPB (Oxford) 23:587-594
96. Kennedy NA, Heap GA, Green HD, Hamilton B, Bewshea C, Walker GJ, Thomas A, Nice R, Perry MH, Bouri S, Chanchlani N, Heerasing NM, Hendy P, Lin S, Gaya DR, Cummings JRF, Selinger CP, Lees CW, Hart AL, Parkes M, Sebastian S, Mansfield JC, Irving PM, Lindsay J, Russell RK, McDonald TJ, McGovern D, Goodhand JR, Ahmad T (2019) Predictors of anti-TNF treatment failure in anti-TNF-naive patients with active luminal Crohn's disease: a prospective, multicentre, cohort study. Lancet Gastroenterol Hepatol 4:341-353
97. Manz M, Vavricka SR, Wanner R, Lakatos PL, Rogler G, Frei P, Safroneeva E, Schoepfer AM (2012) Therapy of steroid-resistant inflammatory bowel disease. Digestion 86 Suppl 1:11-15
98. Burnstock G, Boeynaems JM (2014) Purinergic signalling and immune cells. Purinergic Signal 10:529-564
99. Álvarez-Sánchez N, Cruz-Chamorro I, Díaz-Sánchez M, Lardone PJ, Guerrero JM, Carrillo-Vico A (2019) Peripheral CD39-expressing T regulatory cells are increased and associated with relapsing-remitting multiple sclerosis in relapsing patients. Sci Rep 9:2302
100. Morianos I, Trochoutsou AI, Papadopoulou G, Semitekolou M, Banos A, Konstantopoulos D, Manousopoulou A, Kapasa M, Wei P, Lomenick B, Belaidi E, Kalamatas T, Karageorgiou K, Doskas T, Sallusto F, Pan F, Garbis SD, Quintana FJ, Xanthou G (2020) Activin-A limits Th17 pathogenicity and autoimmune neuroinflammation via CD39 and CD73 ectonucleotidases and Hif1-α-dependent pathways. Proc Natl Acad Sci U S A 117:12269-12280

101. Idzko M, C KA, Müller T, Dürk T, Grimm M, Baudiß K, Vieira RP, Cicko S, Boehlke C, Zech A, Sorichter S, Pelletier J, Sévigny J, Robson SC (2013) Attenuated allergic airway inflammation in Cd39 null mice. Allergy 68:472-480
102. Zacca ER, Amezcua Vesely MC, Ferrero PV, Acosta CDV, Ponce NE, Bossio SN, Mussano E, Onetti L, Cadile I, Acosta Rodríguez EV, Montes CL, Gruppi A (2021) B cells from Patients with Rheumatoid Arthritis Show Conserved CD39-Mediated Regulatory Function and increased CD39 Expression After Positive Response to Therapy. J Mol Biol 433:166687
103. Jin X, Zhang C, Gong L, Li H, Wang Y, Li Q, Li H (2019) Altered expression of CD39 on memory regulatory T cells in type 1 diabetes patients. J Diabetes 11:440-448
104. Longhi MS, Harshe R, Xie A, Zhang H, Robles RJ, Mukherjee S, Vuerich M, Moss AC, Robson SC (2019) Endogenous antisense RNA and dysregulation of CD39 expression in inflammatory bowel disease. The Journal of Immunology 202:182.110-182.110
105. Mascanfroni ID, Yeste A, Vieira SM, Burns EJ, Patel B, Sloma I, Wu Y, Mayo L, Ben-Hamo R, Efroni S, Kuchroo VK, Robson SC, Quintana FJ (2013) IL-27 acts on DCs to suppress the T cell response and autoimmunity by inducing expression of the immunoregulatory molecule CD39. Nature immunology 14:1054–1063
106. Harshe RP, Xie A, Vuerich M, Frank LA, Gromova B, Zhang H, Robles RJ, Mukherjee S, Csizmadia E, Kokkotou E, Cheifetz AS, Moss AC, Kota SK, Robson SC, Longhi MS (2020) Endogenous antisense RNA curbs CD39 expression in Crohn's disease. Nat Commun 11:5894
107. Savio LEB, de Andrade Mello P, Figliuolo VR, de Avelar Almeida TF, Santana PT, Oliveira SDS, Silva CLM, Feldbrügge L, Csizmadia E, Minshall RD, Longhi MS, Wu Y, Robson SC, Coutinho-Silva R (2017) CD39 limits P2X7 receptor inflammatory signaling and attenuates sepsis-induced liver injury. J Hepatol 67:716-726
108. Enjyoji K, Sévigny J, Lin Y, Frenette PS, Christie PD, Esch JS, Imai M, Edelberg JM, Rayburn H, Lech M, Beeler DL, Csizmadia E, Wagner DD, Robson SC, Rosenberg RD (1999) Targeted disruption of cd39/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation. Nature medicine 5:1010–1017
109. Schaefer U, Machida T, Broekman MJ, Marcus AJ, Levi R (2007) Targeted deletion of ectonucleoside triphosphate diphosphohydrolase 1/CD39 leads to desensitization of pre- and postsynaptic purinergic P2 receptors. J Pharmacol Exp Ther 322:1269-1277
110. Peng ZW, Rothweiler S, Wei G, Ikenaga N, Liu SB, Sverdlov DY, Vaid KA, Longhi MS, Kuang M, Robson SC, Popov YV (2017) The ectonucleotidase ENTPD1/CD39 limits biliary injury and fibrosis in mouse models of sclerosing cholangitis. Hepatol Commun 1:957-972
111. Shuai C, Xia GQ, Yuan F, Wang S, Lv XW (2021) CD39-mediated ATP-adenosine signalling promotes hepatic stellate cell activation and alcoholic liver disease. Eur J Pharmacol 905:174198
112. Li XY, Moesta AK, Xiao C, Nakamura K, Casey M, Zhang H, Madore J, Lepletier A, Aguilera AR, Sundarrajan A, Jacoberger-Foissac C, Wong C, Dela Cruz T, Welch M, Lerner AG, Spatola BN, Soros VB, Corbin J, Anderson AC, Effern M, Hölzel M, Robson SC, Johnston RL, Waddell N, Smith C, Bald T, Geetha N, Beers C, Teng MWL, Smyth MJ (2019) Targeting CD39 in Cancer Reveals an Extracellular ATP- and Inflammasome-Driven Tumor Immunity. Cancer Discov 9:1754-1773
113. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ (2010) Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363:711-723
114. Patnaik A, Kang SP, Rasco D, Papadopoulos KP, Elassaiss-Schaap J, Beeram M, Drengler R, Chen C, Smith L, Espino G, Gergich K, Delgado L, Daud A, Lindia JA, Li XN, Pierce RH, Yearley JH, Wu D, Laterza O, Lehnert M, Iannone R, Tolcher AW (2015) Phase I Study of Pembrolizumab (MK-3475; Anti-PD-1 Monoclonal Antibody) in Patients with Advanced Solid Tumors. Clin Cancer Res 21:4286-4293

115. Sun X, Wu Y, Gao W, Enjyoji K, Csizmadia E, Müller CE, Murakami T, Robson SC (2010) CD39/ENTPD1 Expression by CD4+Foxp3+ Regulatory T Cells Promotes Hepatic Metastatic Tumor Growth in Mice. Gastroenterology 139:1030–1040
116. Sun X, Han L, Seth P, Bian S, Li L, Csizmadia E, Junger WG, Schmelzle M, Usheva A, Tapper EB, Baffy G, Sukhatme VP, Wu Y, Robson SC (2013) Disordered purinergic signaling and abnormal cellular metabolism are associated with development of liver cancer in Cd39/ENTPD1 null mice. Hepatology (Baltimore, Md) 57:205–216
117. Feng L, Sun X, Csizmadia E, Han L, Bian S, Murakami T, Wang X, Robson SC, Wu Y (2011) Vascular CD39/ENTPD1 directly promotes tumor cell growth by scavenging extracellular adenosine triphosphate. Neoplasia (New York, N 13:206–216
118. Perrot I, Michaud HA, Giraudon-Paoli M, Augier S, Docquier A, Gros L, Courtois R, Déjou C, Jecko D, Becquart O, Rispaud-Blanc H, Gauthier L, Rossi B, Chanteux S, Gourdin N, Amigues B, Roussel A, Bensussan A, Eliaou JF, Bastid J, Romagné F, Morel Y, Narni-Mancinelli E, Vivier E, Paturel C, Bonnefoy N (2019) Blocking Antibodies Targeting the CD39/CD73 Immunosuppressive Pathway Unleash Immune Responses in Combination Cancer Therapies. Cell Rep 27:2411-2425.e2419
119. Moesta AK, Li X-Y, Smyth MJ (2020) Targeting CD39 in cancer. Nature Reviews Immunology 20:739-755
120. Lavoie EG, Fausther M, Kauffenstein G, Kukulski F, Künzli BM, Friess H, Sévigny J (2010) Identification of the ectonucleotidases expressed in mouse, rat, and human Langerhans islets: potential role of NTPDase3 in insulin secretion. American journal of physiology Endocrinology and metabolism 299:E647-656
121. Vongtau HO, Lavoie EG, Sévigny J, Molliver DC (2011) Distribution of ecto-nucleotidases in mouse sensory circuits suggests roles for nucleoside triphosphate diphosphohydrolase-3 in nociception and mechanoreception. Neuroscience 193:387-398
122. Suzuki Y, Katagiri H, Wang T, Kakisaka K, Kume K, Nishizuka SS, Takikawa Y (2016) Ductular reactions in the liver regeneration process with local inflammation after physical partial hepatectomy. Lab Invest 96:1211-1222
123. Puche JE, Lee YA, Jiao J, Aloman C, Fiel MI, Muñoz U, Kraus T, Lee T, Yee Jr. HF, Friedman SL (2013) A novel murine model to deplete hepatic stellate cells uncovers their role in amplifying liver damage in mice. Hepatology 57:339-350
124. Iwaisako K, Jiang C, Zhang M, Cong M, Moore-Morris TJ, Park TJ, Liu X, Xu J, Wang P, Paik Y-H, Meng F, Asagiri M, Murray LA, Hofmann AF, Iida T, Glass CK, Brenner DA, Kisseleva T (2014) Origin of myofibroblasts in the fibrotic liver in mice. Proceedings of the National Academy of Sciences of the United States of America 111:E3297-3305
125. Vlajkovic SM, Housley GD, Thorne PR, Gupta R, Enjyoji K, Cowan PJ, Charles Liberman M, Robson SC (2009) Preservation of cochlear function in Cd39 deficient mice. Hear Res 253:77-82
126. Chiu DK, Tse AP, Xu IM, Di Cui J, Lai RK, Li LL, Koh HY, Tsang FH, Wei LL, Wong CM, Ng IO, Wong CC (2017) Hypoxia inducible factor HIF-1 promotes myeloid-derived suppressor cells accumulation through ENTPD2/CD39L1 in hepatocellular carcinoma. Nat Commun 8:517
127. Moon JO, Welch TP, Gonzalez FJ, Copple BL (2009) Reduced liver fibrosis in hypoxia-inducible factor-1alpha-deficient mice. Am J Physiol Gastrointest Liver Physiol 296:G582-592
128. Copple BL, Bai S, Moon JO (2010) Hypoxia-inducible factor-dependent production of profibrotic mediators by hypoxic Kupffer cells. Hepatol Res 40:530-539
129. Cui L, Xue H, Wen Z, Lu Z, Liu Y, Zhang Y (2020) Prognostic roles of metabolic reprogramming-associated genes in patients with hepatocellular carcinoma. Aging (Albany N Y) 12:22199-22219
130. Chen Q, Li F, Gao Y, Xu G, Liang L, Xu J (2020) Identification of Energy Metabolism Genes for the Prediction of Survival in Hepatocellular Carcinoma. Front Oncol 10:1210
131. Wu C, Rao X, Lin W (2021) Immune landscape and a promising immune prognostic model associated with TP53 in early-stage lung adenocarcinoma. Cancer Med 10:806-823

132. Shukla V, Zimmermann H, Wang L, Kettenmann H, Raab S, Hammer K, Sévigny J, Robson SC, Braun N (2005) Functional expression of the ecto-ATPase NTPDase2 and of nucleotide receptors by neuronal progenitor cells in the adult murine hippocampus. J Neurosci Res 80:600-610
133. Vandenbeuch A, Anderson CB, Parnes J, Enjyoji K, Robson SC, Finger TE, Kinnamon SC (2013) Role of the ectonucleotidase NTPDase2 in taste bud function. Proc Natl Acad Sci U S A 110:14789-14794
134. Braun N, Sevigny J, Robson SC, Hammer K, Hanani M, Zimmermann H (2004) Association of the ecto-ATPase NTPDase2 with glial cells of the peripheral nervous system. Glia 45:124-132
135. Belcher SM, Zsarnovszky A, Crawford PA, Hemani H, Spurling L, Kirley TL (2006) Immunolocalization of ecto-nucleoside triphosphate diphosphohydrolase 3 in rat brain: implications for modulation of multiple homeostatic systems including feeding and sleep-wake behaviors. Neuroscience 137:1331-1346
136. Gampe K, Haverkamp S, Robson SC, Gachet C, Hüser L, Acker-Palmer A, Zimmermann H (2015) NTPDase2 and the P2Y1 receptor are not required for mammalian eye formation. Purinergic Signal 11:155–160
137. McCoy E, Street S, Taylor-Blake B, Yi J, Edwards M, Wightman M, Zylka M (2014) Deletion of ENTPD3 does not impair nucleotide hydrolysis in primary somatosensory neurons or spinal cord. F1000Research 3:163
138. Brinkman DJ, Ten Hove AS, Vervoordeldonk MJ, Luyer MD, de Jonge WJ (2019) Neuroimmune Interactions in the Gut and Their Significance for Intestinal Immunity. Cells 8:670
139. Grubišić V, McClain JL, Fried DE, Grants I, Rajasekhar P, Csizmadia E, Ajijola OA, Watson RE, Poole DP, Robson SC, Christofi FL, Gulbransen BD (2020) Enteric Glia Modulate Macrophage Phenotype and Visceral Sensitivity following Inflammation. Cell Rep 32:108100
140. Grubišić V, Perez-Medina AL, Fried DE, Sévigny J, Robson SC, Galligan JJ, Gulbransen BD (2019) NTPDase1 and -2 are expressed by distinct cellular compartments in the mouse colon and differentially impact colonic physiology and function after DSS colitis. Am J Physiol Gastrointest Liver Physiol 317:G314-g332
141. Lin JH, Takano T, Arcuino G, Wang X, Hu F, Darzynkiewicz Z, Nunes M, Goldman SA, Nedergaard M (2007) Purinergic signaling regulates neural progenitor cell expansion and neurogenesis. Dev Biol 302:356-366
142. Jiang ZG, Wu Y, Csizmadia E, Feldbrügge L, Enjyoji K, Tigges J, Toxavidis V, Stephan H, Müller CE, Müller CE, McKnight CJ, Moss A, Robson SC (2014) Characterization of circulating microparticle-associated CD39 family ecto-nucleotidases in human plasma. Purinergic Signal 10:611–618

Danksagung

Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

Berlin, 30.01.2022

……. ……..

Datum Unterschrift