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Falk Symposium 181

Innate Immunity in Gastrointestinal Disorders: Basic and Therapeutic Concepts

February 8 – 9, 2012
The Westin Grand Hotel
Munich, Germany

Abstracts
Poster Abstracts
Falk Symposium 181

INNATE IMMUNITY IN GASTROINTESTINAL DISORDERS: BASIC AND THERAPEUTIC CONCEPTS

Munich (Germany)
February 8 – 9, 2012

Scientific Organization:
R.S. Blumberg, Boston (USA)
S. Endres, Munich (Germany)
A. Kaser, Cambridge (Great Britain)
M.P. Manns, Hannover (Germany)
H. Tilg, Hall/Innsbruck (Austria)
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Liver disease and innate immunity – Basic and therapeutic concepts

Session I

Autoimmune liver disease and PBC: From animal models to innovative therapies
The liver as an immune organ

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The liver is known for its unique ability to regulate adaptive immune responses against antigens presented first in the liver as a consequence of antigen expression by liver-resident cells or of uptake circulating soluble antigens. Different cell populations of the liver contribute to its unique microenvironment and function as tolerogenic antigen presenting cells. A particularly prominent liver resident antigen-presenting cell population is the Liver Sinusoidal Endothelial Cells (LSEC). LSEC are scavenger endothelial cells, i.e. are among the most active cells in endocytosis of the entire body. These cells take up circulating antigens and cross-present them to naïve CD8 T cells. Although antigen-presenting LSEC have almost the same efficiency as dendritic cells in terms of stimulation and expansion of naïve CD8 T cells, such stimulation of naïve CD8 T cells by LSEC leads to a state of T cell non-responsiveness towards TCR stimulation. Mechanistically, LSEC mediate this T cell non-responsiveness through mutual interaction with CD8 T cells via the co-inhibitory molecules B7H1 and PD1. Interestingly, LSEC-mediated T cell non-responsiveness is carefully regulated through predominant co-inhibitory over stimulatory signaling processes and is ultimately governed by the lack of IL-2 expression in T cells. It is important to note that LSEC-induced CD8 T cells are not subject to clonal deletion as is typically observed for peripheral immune tolerance. Here, we will discuss novel insights into the relevance of LSEC-induced CD8 T cells for subsequent immune responses and discuss the role of the liver in regulation of immune responses towards circulating antigens.
Animal models of autoimmune liver disease – What is relevant for immune-mediated liver disease?

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Autoimmune hepatitis (AIH) is a chronic autoimmune inflammation of the liver usually requiring life-long immunosuppression. Steroids and azathioprine are the standard therapy, but the therapy is accompanied by strong side-effects. Due to the fact that AIH is often recognized during late course of disease, it is difficult to obtain knowledge about the immunological mechanisms responsible for initiation of the disease. Current AIH models were helpful for understanding and modulating liver immune responses but are not suited to study mechanisms in chronic AIH or to develop new therapies. While transgenic AIH models deal with short-term hepatitis, models with natural antigens are either self limited or have unknown target antigens.

Therefore, new animal models with defined onset of AIH and a standard course of the disease are essential for a more defined understanding of the disease and its pathophysiology.

I) In a first study we attempted to develop a new mouse model of chronic AIH by combining a danger signal with a heterologous human self-antigen within a genetically predisposed host. To this end we generated adenoviral constructs expressing common autoantigens of human AIH showing 80% homology to the murine protein. Following an acute phase of hepatitis with elevated levels of liver transaminases and transient gene expression within the liver, we could show chronic evolving liver-specific autoimmune responses after the infection of non-obese diabetic (NOD) mice with an adenovirus expressing the human formiminotransferase cyclodeaminase (FTCD), a common autoantigen of AIH type-2. This supports the notion that the induction of AIH is linked to environmental as well as genetic factors. Chronic disease was confirmed by massive leukocyte infiltrates which were progressing over time. The mice also developed substantial periportal fibrosis. In parallel we detected a break of humoral tolerance with hypergammaglobulinemia and antigen-specific autoantibodies. Additionally break of humoral tolerance could be achieved by using other non-predisposed mouse strains or other heterologous antigens like the human CYP2D6 and SLA, two important autoantigens of AIH, despite an absent chronic disease.

Regarding the initiation of AIH we could demonstrate that i) Tregs prevent the initiation of AIH, ii) a molecular danger signal in combination with a self-antigen is needed to break tolerance and iii) that molecular similarity is sufficient to break tolerance against liver tissue and that molecular identity is not needed.

Furthermore we identified CD4+ T cells to be the driving force of AIH induction by the detection of IFN-γ producing antigen-specific T cells using an ELISPOT assay and a successful transfer of the disease by CD4+ T cells into immune-deficient syngeneic hosts. Moreover we detected a local increase in NK cells as well as regulatory T cells (Tregs).

In summary, the new mouse model of AIH can be used as a preclinical model of AIH since it shares many characteristics of the human disease and shares many mechanisms with the human disease like the association of genetic and environmental factors with the induction of AIH. Like in humans prednisolone leads to a remission of
the chronic disease. The model will be helpful to test new therapeutic intervention of AIH with less side-effects and improved responsiveness and for investigating the function of Tregs, NK cells within disease development.

II) Predisposition to autoimmune diseases like type I diabetes and multiple sclerosis has been linked to defects in negative thymic selection. Likewise AIH was seen in 20% of patients with an autoimmune polyendocrine syndrome type 1 (APS-1). APS1 is caused by genetic aberrations of the autoimmune regulator (Aire) gene and manifests as spontaneous autoimmunity against multiple organs. We investigated the impact of the genetic background and the predisposition for onset of AIH in the murine model of AIRE knock-out mice. As reported for other autoimmune diseases in the mouse we could demonstrate that the genetic background has an impact on the manifestation and severity of AIH. We found that Balb/c mice with truncated Aire at exon 2 (Aire-Δex2) developed an AIH comparable to those seen in APS-1 patients whereas the symptoms were less pronounced on other genetic backgrounds. Furthermore, the Aire mutation itself seems to be important for the development of AIH, as Balb/c animals with an exon 8 knock-out (Aire-Δex8) did not develop an AIH.

In the Aire-Δex2 model, AIH is characterised by periportal liver infiltrates, which was closely correlated with liver-specific auto-antibodies. In contrast to other autoimmune diseases in APS-1 patients the hepatic immune response is not focussed on single autoantigens but is rather multispecific. This is reminiscent of AIH-Type I patients in which no liver-specific autoantigen could be identified thus far. The hepatic infiltrates of Balb/c Aire-Δex2 animals suffering from AIH have a show significantly increased B cell and Treg proportions. More importantly the hepatitis can be treated by adoptive transfer of CD4+ T cells. Experiments with subpopulations revealed that CD4+Foxp3+ Tregs are responsible for this treatment effect.

In conclusion the new animal models are valuable tools to understand the pathophysiology of AIH. More important they offer the possibility to develop new therapeutic strategies to avoid chronic immunosuppression and to define therapeutic alternatives for patients not responding to conventional therapy.
Autoimmune hepatitis – New guidelines, new therapies

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Autoimmune hepatitis was the first chronic liver disease with a favourable response to drug therapy and a dismal prognosis when left untreated. A timely diagnosis before cirrhosis develops, the avoidance of immunosuppressant side effect, non-responders to standard induction therapy, and adherence to therapy are the greatest challenges.

An established and recently simplified revised scoring system allows for a reproducible and standardized approach to diagnosing AIH in a scientific context and is often employed in clinical practice. The use and interpretation of sero-immunological and molecular biological tests discriminates AIH from other etiologies of chronic hepatitis, i.e. chronic viral infection as the most common cause of chronic hepatitis worldwide. The diagnosis relies on a combination of indicative features of AIH and the exclusion of other causes of chronic liver diseases. An initial liver biopsy specimen is required for diagnostic purposes and for grading and staging. A specific feature of AIH is the association of extrahepatic immune-mediated syndromes.

The indication for treatment is present in patients with established AIH, elevations of aminotransferase activities (ALT, AST), an elevation of serum immunoglobulin G and histological evidence of interface hepatitis or necroinflammatory activity. This is incorporated into 2010 guideline update of the American Association for the Study of the Liver (AASLD).

Since its original description in 1950 and first treatment studies the basic therapeutic strategy of inducing remission with steroids and azathioprine has not been modified in principle. Alternative immunosuppressive drugs have been tested in small series and include transplant immunosuppressants. A recent large multicenter prospective treatment trial suggests that budesonide may offer an alternative in non-cirrhotic AIH patients capable of minimizing unwanted steroid effects. The ultimate treatment approach upon drug treatment failure is liver transplantation. Only 4% of transplant candidates are transplanted for AIH but the risk for graft loss because of recurrence has to be considered and recurrent AIH treated after transplantation.

Suggested readings:


**What's new in primary biliary cirrhosis?**

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There have been significant advances both in humans and experimental models that relate to the etiopathogenesis of primary biliary cirrhosis. Many of these advances are based on the rigorous definition of the antimitochondrial response, the serologic signature of PBC. First, it is well established that AMA are directed against members of the 2-oxoacid dehydrogenase complexes (2-OADC), among which the major epitopes are within the lipoylated domains of the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2). Second, autoreactive CD4+ and CD8+ T cells can be detected in PBC peripheral blood, regardless of the AMA status, and the infiltration of autoreactive T cells in the liver and periductular spaces is one of the most prominent immune features. Autoreactive T cells of both subtypes recognize PDC-E2 sequences overlapping with the AMA epitopes. An increase in cytotoxic T cell precursors in the blood in the early stages of the disease compared to the advanced ones and a 10-fold increase of specific liver CD8+ T cells compared to peripheral blood have been demonstrated. Third, additional data on the immunobiology components of PBC autoimmunity has been recently obtained in CD4+CD25high natural regulatory T cells which appear to be numerically reduced in PBC. PBC bile duct cells manifest unique features during apoptosis while co-culture experiments do not support a direct role for these cells in determining their immune-mediated injury. Apoptotic cells are phagocytosed by BECs and consequently are an exogenous source of autoantigens in cholangiocytes, possibly through anti-CD16. As a result, the impact of putative changes in apoptosis and autophagy specific to BEC remains to be fully determined in PBC. Fifth, the innate immune compartment has been recently investigated in PBC with promising results. PBC monocytes manifest an increased response to pathogen associated stimuli, as indicated by higher levels of pro-inflammatory cytokines. Further, the hyper-IgM associated with PBC is secondary to an aberrant innate immune response, potentially induced by stimulation of toll like receptor 9 by bacterial CpG-B.

The female preponderance may hold an important key to PBC etiology. X-linked genes determine gender-related characteristics at different levels while also regulating the immune function, particularly to maintain tolerance. Major X chromosome defects such as those leading to Turner’s syndrome or premature ovarian failure are commonly characterized by autoimmune comorbidities (particularly thyroid disease) and, less frequently, cholestasis. Our group first determined a significantly higher frequency of monosomy of the X chromosome in peripheral leukocytes (particularly those of the adaptive immune response, i.e. T and B cells) in women PBC compared to age-matched control women. Monosomy frequency correlated with age in all three groups, as expected but monosomic cells were not microchimeric cells. We further demonstrated that the X loss in PBC affected was not random but affected more frequently one parentally-inherited chromosome.
Several key animal models of autoimmune cholangitis have now been described. First, a genomic variant of the non obese diabetic (NOD) mouse (NOD.c3c4) has been observed to manifest autoimmune cholestasis with AMA and ANA positivities in 50–60% and 80–90%, respectively. Liver histology demonstrated portal lymphocyte infiltration with chronic non-suppurative cholangitis and PBC-like granulomas. Second, a dominant negative form of transforming growth factor-β (TGFβ) receptor II (dnTGFβRII) mouse develop serum AMA in 100% of mice. The TGFβ receptor II regulates lymphocyte activation and the appearance of PBC in this model suggests a specific condition of T cells with impaired TGFβ signaling in the presence or absence of B cells is involved. Third, the knockout of interleukin 2 receptor α leads to a murine phenotype with 100% serum AMA positivity, 80% serum ANA positivity, and portal lymphocyte infiltration and vanishing bile ducts. This model is of particular interest based on the report of autoimmune cholangitis in a pediatric case of IL2Rα deficiency. Fourth, Ae2a,b also develop autoimmune phenomenon and a PBC-like disease. Finally, immunization of mice with chemical xenobiotics has also been shown to lead to a PBC-like disease.

These data and observations will be put in the context of the key mechanisms, including the role of TLRs in modulating these responses.
Session II

Innate immunity: Key role in liver disorders
Genetics and the etiopathogenesis of primary sclerosing cholangitis

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Primary sclerosing cholangitis (PSC) is a chronic and severe inflammatory disease leading to fibrotic bile duct destruction and in most cases liver cirrhosis. As in other complex inflammatory diseases, the sibling risk of PSC is approximately ten times that of the general population. Recent genome-wide association studies have consistently identified several genetic susceptibility loci. The overlap of these loci with susceptibility loci in other chronic inflammatory diseases is considerable, and offers intriguing opportunities for transfer of pathogenetic knowledge and potentially treatment options. In the lecture a review of the present knowledge on PSC genetics will be given with a particular emphasis on this overlap. Preliminary data from the ongoing Immunochip project in which approximately 4,300 PSC patients and 26,000 healthy controls have been genotyped for known inflammatory risk loci will be shown. The clinical relevance of the risk loci will be discussed, as well as the potential importance of genetics in explaining the variable disease behavior in PSC, including the occurrence of cholangiocarcinoma.
Primary sclerosing cholangitis – New approaches to diagnosis, surveillance and treatment

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Primary sclerosing cholangitis (PSC) is a chronic inflammatory cholangiopathy with unknown etiology, frequently associated with inflammatory bowel disease (IBD) with peculiar features (PSC-IBD), often leading to end stage liver disease requiring liver transplantation (LTx) or leading to hepatobiliary or colorectal cancer (CRC) reflecting the lack of effective medical therapy for this disorder. Consequently, PSC represents a potentially fatal disease with poor prognosis (medium survival 12 years; 10-year survival approximately 65% with considerable inter-individual variation). Diagnosis, surveillance and therapy of PSC represent major clinical challenges and PSC has been very adequately referred to as one of the last remaining “black boxes” in Hepatology.

Complex pathogenesis: The association with HLA and non-HLA haplotypes and the presence of autoantibodies suggests a role for immune-mediated mechanisms, although immunosuppressive therapeutic strategies are not effective in classic PSC. The strong clinical association between PSC and IBD led to pathogenetic concepts, in which translocation of bacterial products and homing of gut-primed memory T lymphocytes from the inflamed gut plays a central role. Genetically or chemically altered bile composition induces sclerosing cholangitis in a number of animal models (“toxic bile concept”). A central role for vascular injury with bile duct ischemia is supported by animal models of endothelial cell injury showing close morphological similarities with human PSC.

Heterogeneous clinical presentation and diagnostic challenges: PSC shows considerable heterogeneity in its clinical manifestation (i.e., large duct versus small duct PSC, presence or absence of concomitant IBD), disease progression, risk for malignancy and response to therapy. Diagnostic workup in PSC requires exclusion of secondary causes/sclerosing cholangitis (SSC). However, the distinction of PSC from SSC may be challenging due to the similar morphological appearances. Over the past years, several newly identified forms of SSC have been separated from “classic PSC”. This begs for the question whether PSC represents a “mixed bag” of diseases of different – currently unrecognized – etiologies. Moreover, it needs to be kept in mind that part of the identified pathomechanisms (e.g., ischemia, IgG4-mediated immune phenomena) in SSC may also be involved in the pathogenesis of classic PSC. The growing list of secondary causes and diseases mimicking or even overlapping with PSC (e.g., IgG4-associated sclerosing cholangitis), frequently causes problems in clear-cut discrimination from classic PSC. Moreover, features of autoimmune hepatitis are present in 6–9% of adult PSC patients. Cholangiography (MRCP) remains the diagnostic “gold-standard”, while liver biopsy and autoantibody testing have no routine role. ERCP is reserved for endoscopic therapy of dominant strictures (developing in up to 50% of patients) and exclusion of malignancy.
**Surveillance for malignancy:** PSC is associated with a markedly increased risk for hepatobiliary malignancies, mainly cholangiocellular carcinoma (CCA) with a cumulative life time incidence of 10–15%, followed by hepatocellular and gallbladder carcinoma in up to 2% each. Approximately half of the CCA cases are diagnosed at the time of or within the first year after the diagnosis of PSC with a subsequent yearly incidence rate of 0.5–1.5%. The distinction between benign dominant biliary strictures and intraductal CCA is challenging and requires extensive diagnostic work-up. Despite being more often benign in nature any dominant stricture in PSC should be principally suspicious of CCA which is present in 5–20% of all dominant strictures. Tumor markers such as CA19-9 (in conjunction with CEA) may be useful for diagnosis of CCA, although cholestasis per se results in non-specific elevations. The imaging technique of choice in case of suspected CCA is contrast-enhanced MRI and MRCP, followed by CT with supplementary information on lymph node involvement and extrahepatic growth. CCA often grows longitudinally with perineural and perivascular invasion, explaining the limited yield of imaging. PET-CT has no significant advantages over CT. Brush cytology via ERC or PTC has poor sensitivity but excellent specificity. In addition to conventional cytology, FISH (fluorescent in situ hybridization) for chromosomal abnormalities or DIA (Digital Image Analysis) can improve the diagnostic accuracy. Recent reports suggest that proteomic analysis of bile could discriminate between CCA and benign strictures. Since patients with coexistent PSC-IBD have a markedly increased risk for CRC compared to UC patients without PSC, surveillance colonoscopy at 1- to 2-year intervals is recommended. CRC in PSC is typically localized in the right-sided proximal colon.

**Lack of effective medical therapy:** Currently no established medical therapy exists which prolongs (LTx free) survival in PSC. UDCA (15–20 mg/d) improves serum liver tests and surrogate markers of prognosis, but has no proven benefit on survival. A RCT with high-dose UDCA (28–30 mg/kg/d) even showed reduced survival free of liver transplantation compared to the placebo. Current data therefore do not yet allow a recommendation for the general use of UDCA in PSC. UDCA (in standard dosage) may reduce the risk of colorectal dysplasia/malignancy and possibly also reduces the risk of CCA, but the evidence is limited and comes from observational studies. Corticosteroids and other immunosuppressants have no benefical effect in PSC, but have a role for treatment of a PSC-AIH overlap syndrome and IgG4-associated cholangitis which always need to be considered in the differential diagnosis of PSC.

Future therapeutic options may include bile acid receptor/farnesoid X receptor (FXR) agonists (successfully tested in PBC) and 24-norursodeoxycholic acid, a side-chain modified UDCA derivate resistant to amidation which undergoes cholehepatic shunting. Notably, both therapeutic approaches induce secretion of bicarbonate which counteracts biliary toxicity. norUDCA (but not “conventional” UDCA) reverses sclerosing cholangitis in the Mdr2−/− cholangiopathy model and has anti-inflammatory, anti-proliferative and anti-fibrotic properties. Curcumin reduces liver damage, cholangitis and biliary fibrosis in Mdr2−/− mice by targeting portal myofibroblast proliferation (via pERK1/2 inhibition) and cholangiocellular VCAM-1 expression (in a PPARγ-dependent fashion). In addition, fibrates (e.g. bezafibrate, fenofibrate) with pleiotrophic anti-inflammatory effects and stimulation of biliary phospholipid secretion via the PPARα-MDR3 pathway, could represent an attractive therapeutic strategy.
LTx represents the only curative treatment option in PSC and excellent long-term outcomes with 5- and 10-year survival rate between 70 and 85% have been reported. Apart from end-stage liver disease, LTx may also be considered for patients with intractable pruritus, recurrent bacterial cholangitis and cholangiocyte dysplasia. Recurrence of PSC after LTx is discussed controversially, as several other causes such as ischemic biliary strictures may be nearly indistinguishable from recurrent PSC.

Recommended further reading:


Innate immunity in hepatitis C: Role in disease susceptibility and therapy

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The importance of innate immune responses to control HCV infection has been recognized in recent years. Genes encoding for distinct inhibitory NK cell receptors and their human leukocyte antigen ligands influence resolution of hepatitis C virus (HCV) infection suggesting that inhibitory NK cell interactions are important in determining antiviral immunity. HCV may directly interact with NK cells and inhibit NK cell function by interaction between HCV envelope proteins and CD81 which, however, could not been confirmed in some recent studies using infectious hepatitis C virions. NK cells show an activated phenotype in patients with acute hepatitis C which is also associated with severity of liver disease. In chronic hepatitis C, enhanced TRAIL expression and cytotoxicity of NK cells have been described together with an impaired IFNγ production. This might be explained by early changes in interferon signalling. Treatment with interferon alpha further enhances TRAIL expression on NK cells which is inversely correlated with HCV RNA decline during treatment. We could show that NK cells are able to kill HCV-infected hepatoma cells in a TRAIL-dependent manner and that recognition of hepatoma cells by IFNα-stimulated NK cells is dependent on DNAM-1 (CD226). Ribavirin has been suggested to enhance the effects of IFNα, however, we could not find evidence that ribavirin alters the effects of IFNα on NK cell, neither in vitro nor in vivo. NK cell function is tightly regulated by interaction with other immune cells such as dendritic cells or myeloid-derived suppressor cells which also can show altered functions in HCV infection. Finally, NK cells may serve as regulators for other immune cells and thus influence T cells responses.
Session III

Fatty liver diseases: Innate immunity as driving force for metabolic inflammation?
The intestinal microbiota: A key player in obesity and related disorders?

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The human gut is home to a vast number of microorganisms, especially bacteria, which are estimated to outnumber our human cells by at least an order of magnitude. Thus our human body is an amalgam of eukaryotic and bacterial cells that both affect our metabolism. We have demonstrated that gut microbial ecology is altered in obese mice and humans and that germ-free mice are protected against developing diet-induced obesity. Thus the gut microbiota can be considered an environmental factor that contributes to obesity. Further analyses of germ-free mice have revealed that the gut microbiota affects host metabolism by several mechanisms. One such example of host-microbe cometabolism is biconversion of cholesterol to bile acids, which is essential for cholesterol excretion in feces. Furthermore, bile acids are important signaling molecules binding to different receptors that in turn regulates host metabolism. By detailed characterization of the bile acid metabolism in germ-free and conventionally raised mice we demonstrated that the gut microbiota is not only essential for production secondary bile acids but also for hepatic synthesis of muricholic acids by a FXR/FGF15 dependent mechanism. Accordingly, production of bile acids and other microbial products such as short chain fatty acids may regulate host adiposity and metabolism both directly and indirectly.
Chronic, low grade, inflammatory responses in metabolically active sites such as adipose tissue is a hallmark of chronic metabolic disease, especially obesity. We refer to this metabolically triggered chronic immune response as metaflammation which provides a critical link between obesity and other associated pathologies, such as insulin resistance, type 2 diabetes, hepatosteatosis, cardiovascular disease and other pathologies. In metabolic context, inflammatory cascades involve both metabolic cells such as adipocytes and hepatocytes as well as immune effectors such as macrophages, mast cells, T cells, and eosinophils. The collective activity of these inflammatory networks intersects with insulin receptor signaling at several levels to block insulin action and disrupt glucose and lipid metabolism through several different direct and indirect mechanisms. Our earlier work has identified the activation of inflammatory kinases such as c-Jun N-terminal kinase (JNK) as a critical event seen in all forms of insulin resistance. Our studies in search for intrinsic pathways leading to JNK activation and metabolic dysfunction lead to the discovery of endoplasmic reticulum (ER) dysfunction as a critical mechanism underlying metabolic disease, especially obesity and diabetes. Recently, we identified protein kinase R (PKR) as a potential intersection point between nutrients, organelle dysfunction and metabolic control. In liver tissue, dysfunction of the ER driven by alterations in lipid metabolism and membrane composition is a key mechanism giving rise to fatty liver disease, insulin resistance, and diabetes. Using these systems, we are now pursuing the precise mechanisms by which nutrient sensing and metabolism intersect with immune response and organelle stress and identify the molecular targets of specific nutrients in their ability to engage components of the innate immune response. Here, I will present the latest developments and translational opportunities emerging from these platforms that are applicable to human metabolic diseases.
Innate immunity in NAFLD

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Work in animal models suggests that the onset and progression of NAFLD result, in large part, from innate immune system responses to metabolic stress. Such stress stimulates various types of liver cells to produce tumor necrosis factor (TNF) alpha and interleukin (IL)-6, pro-inflammatory Th1 cytokines. This may explain why mortality from other chronic inflammatory conditions (e.g., atherosclerotic cardiovascular disease and cancer) are increased in individuals with the metabolic syndrome and NAFLD. In contrast, death from liver disease rarely occurs in individuals with NAFLD unless they have developed cirrhosis. Classically, liver fibrosis is thought to result from excessive and sustained exposure to Th2-type cytokines. Assuming the same holds true in NAFLD, then it will be important to understand what transforms the hepatic microenvironment from one of excessive Th1 (proinflammatory/anti-fibrogenic) cytokines in early stage NAFLD, to that of advancing liver fibrosis, where the actions of Th2 (anti-inflammatory/profibrogenic) cytokines, such as TGF beta, IL4 and IL13 predominate.

Liver fibrosis is significantly more common in individuals with nonalcoholic steatohepatitis (NASH) than in those with simple nonalcoholic fatty liver (NAFL). A major difference between NASH and NAFL is hepatocyte death, which is much more prevalent in NASH than NAFL. We've discovered that repair/remodeling responses that are evoked by hepatocyte death contribute to changes in the innate immune system that favor relative over-production of Th2 cytokines within the liver. Dying hepatocytes produce damage associated molecular signals (DAMS) that trigger regenerative mechanisms. Novel evidence demonstrates that Hedgehog (Hh) ligands, key fetal developmental morphogens, are an important type of DAMS produced by ballooned hepatocytes in NASH. Hh ligands unleash a cascade of responses, including the outgrowth of hepatic stellate cells, liver progenitors, and immune cells. These Hh-responsive cells, in turn, collaborate to promote hepatic fibrogenesis by enhancing local production of various fibrogenic factors, including TGFbeta, IL4, IL13, and osteopontin. Targeted inhibition of Hh signaling and osteopontin reduces liver fibrosis in animal models, consistent with the concept that these pathways contribute to fibrosis progression in NAFLD. The notion if further supported by human data, which also reveal strong correlations between the intensity of Hh signaling, osteopontin production, and liver fibrosis severity. Thus, the innate immune system is altered to favor fibrogenic repair as part of a wound healing response that aims to regenerate dead hepatocytes in NAFLD. This discovery helps to explain why liver fibrosis is more common in NASH than simple NAFL.
Innate immunity and alcoholic liver disease

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Innate immunity provides the primary response to danger signals from pathogens or injured host cells and tissues. The cells of the innate immune system include monocytes, macrophages, dendritic cells, neutrophils, natural killer cells and NKT cells that orchestrate innate immune and initiate adaptive immune responses via cell interactions, cytokines, chemokines and other mediators. The most robust and common response of the innate immune system to danger signals is inflammation. In the multifactorial pathophysiology of alcoholic liver disease (ALD), activation of innate immune cells and the inflammatory cascade plays a central role. Recent studies demonstrated that Toll-like Receptors, the sensors of microbial and endogenous danger signals, are expressed and activated in innate immune cells as well as in parenchymal cells in the liver and thereby contribute to ALD. The importance of gut-derived endotoxin and its recognition by TLR4 expressed on innate immune cells and liver parenchymal cells and the specificity of TLR4-induced downstream signaling via the interferon regulator factor 3 (IRF3) has recently been investigated. We have shown that mice deficient in IRF3 or TLR4 expression are protected from alcohol-induced liver steatosis, inflammation and hepatocyte injury. In addition to pathogen-derived danger molecules, the inflammatory cascade can also be activated by endogenous danger signals released from damaged cells. The inflammasome, a multiprotein complex, senses endogenous danger molecules to result in caspase-1-mediated cleavage of IL-1β. Our recent results suggest that inflammasome and caspase-1 activation occur in alcoholic liver disease and that IL-1 significantly contributes to both steatosis and inflammation in the liver in ALD.
Session IV

Fatty liver disease: Histopathology and treatment
NAFLD: What the pathologist can tell the clinician

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The Obvious: Diagnosis, Evaluation of Severity, Clinical Trial Applications

Liver tissue evaluation by light microscopy continues to serve useful roles in current medical practice. The first, and most apparent, is diagnosis. Various hepatic and systemic disease processes do not invoke specific diagnostic serologic markers, such as metabolic syndrome, alcoholic and nonalcoholic fatty liver, and liver tissue evaluation remains key in the assessment of "unexplained" abnormal liver tests. The literature also documents biopsy-proven NAFLD in the presence of normal weight/BMI, transaminase tests, raised autoantibodies (ANA, ASMA, AMA), and hyperferritinemia. Conversely, tissue evaluation has proven that NAFLD may not be the cause in "at-risk" subjects for otherwise unexplained liver test elevations. Liver biopsy remains the only means of diagnosing concurrent NASH with other forms of serologically-diagnosable liver diseases. Finally, liver tissue examination has shed light on the concept of burned-out NASH in otherwise "cryptogenic" cirrhosis (CC). Careful evaluation has also shown that not all CC is NASH, and conversely that NASH may actually retain active lesions in cirrhosis.

NASH, considered the potentially progressive form of fatty liver disease not attributable to alcohol over-use, shares two histopathologic features with the non-progressive lesion of "steatosis": the presence of intrahepatocellular fat predominantly in the form of macrovacuoles and varying forms of lobular inflammation. The inflammatory subtypes present within "steatosis/steatosis with inflammation" have not been well phenotyped. Steatohepatitis, on the other hand, requires more than steatosis and inflammation. An essential component is the presence of a form of hepatocyte damage known as ballooning, the nature of which is being clarified. The pattern of collagen and basement membrane deposition in NASH is unusual in chronic liver disease; in adults, this is in perisinusoidal spaces in acinar zones 3. Progression may remain intraparenchymal, or may involve ductular reaction and portal-based fibrosis. Patterns of NAFLD, NASH and fibrosis may vary substantially in subsets of subjects including bariatric and younger aged subjects.

Grading (lesions of activity) and staging (lesions of fibrosis and vascular remodeling) overlap somewhat with, but are distinct exercises from, diagnosis. Values of structured grading and staging systems include use in clinical trials.

The Not-So-Obvious

Tissue evaluation remains a valuable standard against which noninvasive markers are developed. Biopsy and explant tissue have additionally provided resources for genetic studies and mechanistic insights in human fatty liver disease.
Non-alcoholic fatty liver disease (NAFLD) is increasingly diagnosed worldwide and considered to be the commonest liver disorder in Western countries. It comprises a disease spectrum ranging from simple steatosis (fatty liver), through non-alcoholic steatohepatitis (NASH) to fat with fibrosis and ultimately cirrhosis. Simple steatosis is largely benign and non-progressive, whereas NASH, characterized by hepatocyte injury, inflammation and fibrosis can lead to cirrhosis, liver failure and hepatocellular carcinoma (HCC). NAFLD is strongly associated with obesity, insulin resistance, hypertension and dyslipidemia and is now regarded as the liver manifestation of the metabolic syndrome. Therapeutic strategies can be divided into those directed at components of the metabolic syndrome with potential beneficial liver effects and those directed specifically at the liver. The former group include weight reduction therapies, insulin sensitisers, lipid lowering agents and anti-hypertensives. With respect to weight reduction, recent data from controlled trials suggest that diet and exercise improves NASH, particular in those achieving > 7% weight loss. Obesity surgery has been shown to improve steatosis in all studies and inflammation and fibrosis in some. Insulin sensitisers are the rational choice for patients with NASH and associated diabetes, however, results for metformin have not been convincing and concerns over the safety of glitazones has reduced the initial enthusiasm for their use based on encouraging pilot data. There has been no convincing evidence of any benefit of lipid lowering agents, however, importantly, statin therapy is safe in patients with NASH and should be given for the normal indications. Given the role of the renin-angiotensin system in liver fibrosis, ACE inhibitors and angiotensin II receptor blockers hold most promise as anti-hypertensive agents for patients with NASH and hypertension. Again, pilot data have been encouraging. With respect to more specific liver-directed therapies, there have been promising studies of antioxidants, including betaine and probucol and Vitamin E may improve NASH in adults either alone or taken together with ursodeoxycholic acid. Ursodeoxycholic acid alone appears to have no beneficial effect. As in alcoholic hepatitis, the TNFα lowering agent, pentoxifylline may have beneficial effects on NASH. Liver transplant is successful but disease recurrence rate is high in the absence of treatment of the underlying metabolic syndrome. Promising therapies at present, based largely on animal studies, include probiotics, drugs directed at ER stress and inhibitors of the IKK/NF-κB system.
While the Nobel-Price for Physiology/Medicine went 1908 to Paul Ehrlich (Specificity of Adaptive Immunity) and Iljitsch Metschnikow (Non-specificity of Innate Immunity), Jules Hoffmann (France) and Bruce Beutler (USA) shared half of the 2011 Nobel-Price for their discoveries concerning specific activation of Innate Immunity via Toll-like receptors (TLRs). Medzhitov and Janeway cloned the first human TLR gene (now TLR4) to show 1997 that its overexpression caused cytokine production, while in 1998 Bruce Beutler was first to identify LPS as ligand for TLR4. While humans express 10 germline encoded TLRs (mice 13), numerous TLR binding/activating pathogen-associated and damage-associated molecular pattern (P(D)AMPS) have been characterized. Furthermore, beyond innate immune cells TLRs are expressed on numerous other cell types including the intestinal epithelium (and thus exposed to the intestinal microbiota). We will discuss mechanisms through which the host immune system determines whether a bacterium is a friend or a foe. In the case of “friendly” Bacteroides fragilis its capsula polysaccharide A (PSA) activates via TLR2 regulatory T cells (Tregs) to produce the immune suppressive cytokine IL-10. On the other hand, the unculturable segmented filamentous bacteria (SFBs) activate inflammatory/protective Th-17 and Th-1 T cells to recruit macrophages, neutrophils and to induce antibacterial defensins. Inflammatory bowel diseases (IBDs) are thought to arise owing to a combination of genetic- and environmental factors that result in dysregulated immune responses to gut’s microbiota – and subsequent development of gut inflammation.
Intestinal diseases and innate immunity

Session V

The new world of IBD: About genes, bacteria and viruses
High throughput human gut metagenomics study

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To increase the knowledge of human gut microbiome, metagenomics sequencing is more and more widely performed on many cohorts around the world, focused on different clinical studies like obesity, IBD, diabetes. In BGI, we analyzed 124 MetaHIT and 73 Chinese gut metagenomic samples, and provided a large scale study on reference gene set, genetic variations in population, and association study with Type 2 Diabetes. Analyzing the huge data by Illumina sequencing technology, many biological hypotheses assisted in and benefited from the discovery of data mining, which demonstrated that a large diversity of gut microbiome, the dysbiosis of gut microbiome in Type 2 Diabetic patients, and so on. Besides, BGI has developed many bioinformatics tools which can handle metagenomic profiling, meta-species prediction and association analysis, and also establish a sequence-based microbiology experiment platform, which works on meta-transcriptome, meta-proteome, microbial single-cell sequencing, and bacteria high-throughput isolation, contributing into an incoming personalized medicine research on human gut microbiome.
The genetics universe of Crohn's disease and ulcerative colitis

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In no other common disease have genetic studies so illuminated new pathogenic mechanisms as IBD. As predicted from their close clinical relationship some key susceptibility loci are shared between Crohn’s disease (CD) and ulcerative colitis (UC). The first IBD gene identified by GWAS was IL23R, with variants initially associated with CD but subsequently also with UC – and indeed other clinically related autoimmune conditions ankylosing spondylitis and psoriasis. Subsequent studies have identified association between CD and UC and an ever increasing number of loci encoding IL23/Th17 pathway genes. While intuitively the IBD-associated variants in IL23R might be predicted to exert their effect on adaptive immunity via Th17 pathways, they may also impact innate immunity. For example, Buoncore et al recently reported the accumulation of IL23 responsive innate lymphoid cells and intestinal cells in the colon, the former capable of producing IL17 and interferon-γ and mediating innate colitis in mice. Production of Th17 cytokines by analogous cells in humans appeared higher in colons from IBD cases vs. controls. This pathway now represents a major target in drug development.

The early identification of NOD2 as a susceptibility gene for Crohn’s disease, in the era of genome-wide linkage studies, first catalysed major interest in the role of innate immunity in IBD. This interest has been substantiated in the era of Genome-wide association scanning (GWAS) with the identification of genetic association between Crohn’s disease and variants in two separate autophagy genes, ATG16L1 and IRGM. Prior to the GWAS era this had not been considered relevant to Crohn’s disease pathogenesis; 5 years later and a Google search on ‘Crohn’s autophagy’ produces > 70,000 hits, underlining the interest generated in this pathway.

Since genetics studies first highlighted the contribution of NOD2, ATG16L1 and IRGM numerous studies have explored the functional impact of the Crohn’s disease-associated risk variants. Hugot’s original study identified 3 low frequency variants clustered in the part of the NOD2 gene encoding the leucine rich region, which binds muramyl dipeptide. Extensive resequencing of NOD2 has identified a number of additional rare variants and private mutations associated with Crohn’s disease. A variety of disease-predisposing mechanisms have been proposed for the NOD2 mutations, ranging from defects in viral sensing and reduced mucosal defensin production to abnormal autophagy induction and antigen presentation. As more is understood of NOD2 biology, ever more putative IBD-predisposing mechanisms are revealed – and it may be precisely because of NOD2’s pleiotropic roles in innate immune responses that its mutation exerts such a powerful effect in predisposing to Crohn’s disease.

Recent work has also highlighted the complexity of the contribution made by genetic variation in the autophagy genes ATG16L1 and IRGM. For IRGM the risk alleles are non-coding and appear to affect mRNA transcription or stability. A number of mechanisms have been proposed including disruption of a transcription factor binding site in the IRGM promoter and, intriguingly, alteration of a microRNA binding site by a
synonymous coding variant. The functional impact of the altered production of IRGM has been explored, and particularly correlated with impaired clearance by macrophages of CD-associated adherent-invasive E. coli\(^6\).

Many other genes linked to various components of innate immunity are evident among the >> 100 confirmed IBD susceptibility loci highlighted by GWAS scans, recently published meta-analyses and on-going follow-up studies using Immunochip\(^7,8\). An intriguing finding is that variants in genes linked to epithelial barrier function seem to be specifically associated with UC and not Crohn’s disease - the converse of NOD2 and the autophagy genes which are Crohn’s specific. While many complex explanations might exist, these observations correlate nicely with UC being confined to the superficial layers of the colon, while the transmural inflammation of Crohn’s disease is caused by defects in cellular innate immunity and bacterial handling in the deeper layers of the lamina propria and beyond.

References:

Colitis: Host-microbial detrimentalism?

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Numerous studies of the microbiota that are found throughout the human body are underway with the goal of unraveling the role of microbes in both human health and disease. Inflammatory bowel disease (IBD) is an ideal setting for such studies as disruption of homeostasis between the host immune system and the intestinal microbiota is now a well-accepted contributor to IBD pathogenesis. Using experimental colitis models, we are investigating microbes that may instigate chronic inflammation as well as putative beneficial microbes whose reduced presence may impact not only host response to the microbiota but also the behavior of the endogenous microbiota. Chronic inflammation in the intestine is not only the central pathophysiologic mechanism of IBD but also a key contributor to colorectal cancer. Ongoing work on the colorectal microbiome using experimental models and human tumors will be discussed. Collectively, our studies support the utility of wedding culture-independent and culture-dependent studies with mouse models for defining how the gut microbiota works in concert with the mucosal immune system to shape disease susceptibility for IBD and colorectal cancer.
Could IBD be an infection in a genetically susceptible host?

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Inflammatory bowel disease (IBD) is considered to occur in genetically susceptible individuals. Meta-analyses of genome wide association studies have discovered many new susceptibility loci associated with interesting genes. In European populations with Crohn’s and ulcerative colitis, these studies have found ~99 susceptibility loci in total with 28 loci that overlap between these two major forms of this disease. However, this is not solely a genetic disease. Many of the susceptibility loci are present in high frequency in target populations and the incidence of this disease has been increasing in recent times. Thus, it has long been recognized that environmental factors, potentially including pathogenic infections as well as shifts in commensal microbiota, are also required to trigger disease. Thus a key area for the advancement in our understanding of IBD pathogenesis is the better understanding host susceptibility in the context of interaction with the microbial environment. Improved methods to analyze the commensal microbiota and functionally test its components as well as better methods to define dysbiosis and its potential role in disease have been crucial to improve our understanding of host-microbial interactions that affect disease. Lastly, identification of microbial triggers including viruses in experimental systems of IBD suggests a potential role in IBD. Understanding the precise microbial and immune triggers of IBD in a genetic context will hopefully lead to a better understanding of the pathogenesis of this disease and the discovery of novel therapeutic approaches including vaccines for specific viruses.
Session VI

Focus on innate immunity in IBD
New players in the intestinal innate immune system

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The innate immune system uses germ line encoded pattern recognition receptors (PRRs) to detect microbial pathogens or other forms of danger. Signalling by PRRs induces inflammatory responses and host defense. Bonafide PRRs with signalling capacities that reprogram gene expression include Toll-like receptors, Not-like receptors, RIG-I-like helicases and the recently emerging family of spleen tyrosin kinase (SYK) coupled C-type lectin receptors (CLR). Crucial for CLR mediated innate immunity and inflammation is the adapter protein CARD9. While loss of function mutations in CARD9 lead to immunodeficiencies recent work identified genetic polymorphisms in CARD9 that are associated with human inflammatory diseases. Here we will discuss new insights into the regulation of CARD9 in the innate immune system and how these might relate to inflammatory disease.
The inflammasome in inflammatory bowel disease (IBD)

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IBD is characterized by chronic, relapsing and remitting inflammatory conditions that result from chronic dysregulation of the mucosal immune system in the gastrointestinal tract. Next to TNF-α, the proinflammatory cytokines IL-1β and IL-18 are central players in the pathogenesis of IBD. In response to a variety of microbial components both cytokines are processed via the caspase-1 activating multi-protein complex, the NLRP3 inflammasome. Single nucleotide polymorphisms in the NLRP3 gene region are associated with susceptibility to Crohn’s disease. We investigated the regulation of caspase-1 activation in murine macrophages with genetic deletions of NLRP3 inflammasome components and studied its role in intestinal inflammation using the acute DSS colitis model (Bauer et al. Gut 2010).

Macrophages secreted high levels of IL-1β in a caspase-1-dependent manner in response to DSS exposure. IL-1β secretion was abrogated in macrophages lacking NLRP3, ASC or caspase-1, indicating that DSS activates caspase-1 via the NLRP3 inflammasome. Moreover, IL-1β secretion was dependent on phagocytosis, lysosomal maturation and cathepsins B and L. After oral administration of DSS, NLRP3−/− mice developed a less severe colitis than WT mice and produced lower levels of proinflammatory cytokines in colonic tissue. Interestingly, pharmacological inhibition of caspase-1 with pralnacasan achieved a comparable level of mucosal protection as NLRP3 deficiency.

Interestingly, other groups have reported that NLRP3−/− mice are more prone to DSS colitis pointing to a possible role of the inflammasome in mucosal healing (Zaki et al. Immunity 2010). However, it appears that so far undefined constituents of the intestinal microflora determine whether the inflammasome is protective or detrimental in the inflamed mucosa.

In summary, we and others have identified the NLRP3 inflammasome as a critical regulator of intestinal inflammation in the DSS colitis model. The NLRP3 inflammasome may evolve as a target of novel therapeutics for patients with IBD.
Animal models of IBD

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Inflammatory bowel diseases (IBD) are chronic inflammatory diseases of the intestine of unknown origin. Animal models of IBD have provided new insights in the pathogenesis of chronic gut inflammation and research in animal models has also highlighted new targets for therapy. In addition to established models of colonic inflammation, recent studies have described new models of small bowel inflammation and ileitis. Furthermore, new models of IBD associated cancer have been developed in recent years. In this presentation, animal models of colitis, ileitis and colitis-associated colon cancer will be presented and discussed.

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Inflammation and colorectal cancer

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A causal link between inflammation and cancer has been suspected for a long time and during the last decade several of the underlying molecular mechanisms have been unraveled. In particular colorectal cancer represents a very good example demonstrating the close connection between chronic inflammation and tumorigenesis. On the one hand it is evident that patients suffering from chronic inflammatory bowel disease, especially ulcerative colitis are at increased risk to develop cancer yet on the other hand it has become evident that also sporadic tumor development is associated with an inflammatory reaction. Accordingly, non-steroidal anti-inflammatory drugs can prevent colonic tumor development. Functional animal studies have helped to decipher important molecular and cellular events that interconnect inflammation, tumor micro-environment and carcinogenesis. One of the main signaling pathways in this context is the classical NF-κB activation pathway. During the tumor promotion stage NF-κB has a dual role by either regulating directly the survival of tumorigenic cells or indirectly in immune cells by controlling the transcription of a variety of pro-inflammatory cytokines, which can act in a paracrine manner to stimulate growth of initiated cells. Furthermore, we have obtained evidence for an important role of NF-κB in stem cell expansion during the initiation of tumorigenesis as well as an essential contribution to invasion during the tumor progression stage. In addition to NF-κB another transcription factor has been documented to confer important regulatory functions in epithelial cells regarding the control of apoptosis and proliferation during intestinal tumorigenesis: Stat3. Using loss-of-function and gain-of-function mouse models in an inflammation-associated tumor model, we are able to demonstrate that IL-6 family cytokines control Stat3 induced transcription of a variety of genes important for cell survival and proliferation of tumorigenic cells. Thus, Stat3 and NF-κB comprise the central signaling nodes in intestinal tumorigenesis.
Session VII

Treatment update of IBD
5-ASA: Novel aspects of an old drug

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5-Aminosalicylate derivatives (5-ASAs) have been successfully used for the treatment of mild to moderate Crohn’s disease (CD) and ulcerative colitis (UC) for several decades. While treatment with 5-ASA remains a fundamental strategy for the induction and maintenance therapy in mild to moderate UC, the use of 5-ASA in CD patients is still controversial. Early trials with sulfasalazine in CD had demonstrated benefits for sulfasalazine in colonic involvement, however, controlled trial evidence for the role of sulfasalazine and other 5-ASA formulations as an induction and maintenance therapy for both small bowel and colonic CD is still conflicting and metaanalysis show only a modest therapeutic benefit in unselected patient populations. Nevertheless, oral mesalazine has been demonstrated in controlled trials to be superior to placebo in mild to moderate CD. However, in a recent trial with Eudragit L-coated mesalazine in patients with mild to moderate CD, mesalazine was not inferior to the comparator budesonide, the current standard of care in patients with mild to moderate ileocolic CD, and remission rates over 60% were observed. However, the efficacy of 5-ASA is inferior to systemic corticosteroids in inducing remissions in CD. The maintenance benefits of 5-ASAs in CD appear to be limited to patients in whom remission had been induced with 5-ASA and in some postoperative settings.

In contrast, it is generally accepted that 5-ASA derivatives are standard of care for both induction of remission and maintenance treatment in patients with mild to moderate UC. Topical 5-ASA is the most efficacious treatment in distal UC. Oral 5-ASAs have been shown to be highly effective in inducing and maintaining remission in mild-moderate UC with extensive and also left-sided involvement. Interestingly, combined treatment with oral and rectal application of 5-ASA improves the therapeutic responses in both distal and extended UC.

Recent interest focuses on the optimization of 5-ASA use in both UC and CD. Recent studies assess new dosing schedules, new formulations with different release kinetics and combination of oral and rectal 5-ASAs or combination of 5-ASAs with other drugs like probiotics, immunomodulators and antibiotics. New 5-ASA dosing schedules have demonstrated that patients’ adherence to 5-ASA therapy was improved resulting in at least the same efficacy as 2–4 times daily dosing of 5-ASAs in clinical trials. Recent trials with various slow-release mesalazine formulations in patients with active and quiescent UC have demonstrated that OD dosing of retarded mesalazine was more efficacious than the previously widely accepted and recommended several times daily dosing regimen.

However, a number of unsolved questions remain to be addressed in the future regarding optimal dosing of oral and rectal 5-ASAs as an induction and maintenance agent for both UC and CD. The dose-response and efficacy of 5-ASAs after steroid-or immunomodulator-induced remissions in UC has not been studied systematically. The role of 5-ASA for induction and maintenance treatment of CD needs to be addressed again, as better characterization and selection of patients may help to select patients subgroups that may benefit from treatment with 5-ASAs.
In 1950 the Nobel Prize was awarded for the description of the structure and the function of adrenal cortex hormones, the same year when a German company started the commercial production of cortisone and seven years later, the production of prednisolone. This time marked the beginning of a fundamental change in treatment options for patients with inflammatory bowel diseases, comparable only to the introduction of TNF-alpha-antibodies at the end of the last century. Since then, steroids have been used to control inflammation in flare-ups of Crohn’s disease and ulcerative colitis and due to an unfortunate lack of alternatives also to maintain remission. Although thiopurines and TNF-alpha-antibodies offer an opportunity since many years, reluctance to use these substances is still noticeable in physicians who take care of IBD-patients, mainly due to high costs, a lack of experience and the overestimated fear of side effects of TNF-alpha-antibodies. This is even more remarkable if one takes into account that steroids have much more pronounced side effects in almost every patient. Osteoporosis, hypertension, cataract, diabetes, thrombosis, Cushing-like symptoms, adrenal insufficiency, and psychosis are very frequent in patients on prolonged steroid courses. Furthermore, the use of steroids has been identified as one of the worst outcome predictors in chronically active IBD. If steroids would have to undergo pharmaceutical approval processes nowadays, they would probably not pass phase II trials in IBD. Since the chronic inflammatory character of the IBD necessitates the use of steroid-free long-term medication, the role of steroids is diminishing.
Where do immunosuppressants fit in the treatment algorithm?

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This talk will concentrate on the place of azathioprine (AZA)/6MP in the treatment of luminal Crohn’s disease (CD) because it has been the topic of most recent controversies.

CD is a chronic progressive disease with transition in most patients from inflammatory lesions to destructive complications such as stricture, fistula and abscess. These complications lead to surgery and irreversible bowel damage that in turn leads to loss of gastrointestinal tract function and disability. It is no more acceptable to simply treat symptoms of the disease; instead, the goals of treatment should be to change this natural history and prevent disease progression, bowel damage, and disability.

At present AZA/6MP fits within three main therapeutic strategies: the conventional “step-care”, whereby corticosteroids and immunosuppressants (IS) are prescribed sequentially; an accelerated step-care approach where IS are immediately initiated in combination with a tapering course of steroids and the top-down approach with TNF antagonists in combination with IS. The choice between strategies should be guided by efficacy and safety data and cost-effectiveness. AZA/6MP are superior to placebo for maintenance of clinical remission in patients with CD, however the effect size is modest.

In the conventional step-care, TNF antagonists are reserved for refractory disease or intolerance to conventional therapies. This approach has not been shown to slow or prevent disease progression to bowel damage. The accelerated step-care approach has been shown to be effective in pediatric CD reducing the risk of surgery. Data in adults are still controversial. Finally, the collective results of recent trials are consistent with superior efficacy for combined immunosuppression. As far as safety, the most important complications of immunosuppression are serious infection and malignancies. In the TREAT registry serious infection risk for infliximab alone or in combination with IS was similar, conferring a two-fold increased risk in both cases. Whether the use of TNF antagonists in combination with IS is associated with an increased risk of non-Hodgkin’s lymphoma in adult patients with CD beyond what would be expected from IS therapy alone is unclear. IS therapy with AZA/6MP is clearly associated with an increased risk of non-Hodgkin’s lymphoma. To date, 36 cases of HSTCL have been reported in patients with IBD. All patients had prior or concurrent use of thiopurines for at least 2 years, alone (n = 16) or in combination with TNF antagonists (n = 20). Finally an increased risk of non-melanoma skin cancer has been reported in IBD patients treated with AZA/6MP and rheumatoid arthritis patients on anti-TNFs. It is not known if combo therapy increases the risk. A cost-effectiveness analysis comparing conventional step-care to the early use of AZA/6MP and/or TNF antagonists is lacking in IBD. Interestingly it was shown that in rheumatoid arthritis, very early intervention with conventional DMARDs was cost-effective while the cost-effectiveness of very early intervention with biologics remains uncertain.

Apart from these 3 main strategies, a bridging strategy has also been tested. A short bridge using an induction therapy with infliximab should be avoided but a long bridge may be considered in a selected group of patients identified through a combination of clinical and biological markers.
Finally the choice between strategies is a case by case decision. Population-based studies have shown that the clinical course of CD is heterogeneous and varies widely over time. There is no validated marker of disease progression in CD but some clinical parameters may be used to select patients who merit early intensive therapy. Based on these risk factors, an algorithm of the treatment of luminal CD may be proposed.

References:


Predicting the course of IBD: Light at the end of the tunnel?

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The natural history of Crohn’s disease (CD) and ulcerative colitis (UC) (following diagnosis) varies substantially between patients. For this reason, no single treatment strategy will be suitable for all patients. “Top down” strategies, which pre-emptively use biological or combination therapies, would inevitably overtreat patients who were destined to experience indolent disease without any additional therapy (and thus expose them to the risks and toxicity of unnecessary immunosuppression), whilst “step-up” strategies will expose patients with frequently-relapsing, treatment-refractory disease to potentially avoidable disease-related morbidity and complications whilst ineffective therapies are trialled. There is therefore a major need to be able to reliably predict disease course at diagnosis so that treatment strategies can be appropriately stratified.

In oncology, gene-expression profiling has been used to predict several aspects of tumour behaviour, including response to therapy and risk of metastasis. However, this has been less successfully applied to autoimmune and inflammatory disease – often because heterogeneous cell populations are studied, resulting in signatures being detected that simply reflect compositional differences in the starting material. This can be overcome by analysing purified cell populations. We have previously shown that a prognostic gene-expression signature is detectable in purified CD8+ T-cells in two distinct autoimmune diseases (systemic lupus erythematosus and ANCA-associated vasculitis). These distinct conditions share a relapsing-remitting course driven by immune responses to antigen – in common with CD and UC. More recently we have shown that the same prognostic signature exists in CD and UC and is detectable from diagnosis. This signature did not correlate with contemporaneous clinical, serological or biochemical data, but did significantly associate with prognosis in both conditions. In both disease cohorts, patients enriched for the signature (subgroup “IBD1”) experienced a significantly more aggressive disease course with more frequent flares and a significantly higher incidence of treatment-refractory disease. Accordingly, this suggests that gene-expression profiling may represent the first method by which patients with CD or UC could be stratified at diagnosis according to their future disease course, and represents a major step towards personalised therapy in IBD.
Session VIII

The future of IBD:
Genetics meets Therapeutics
New IBD therapies on the horizon

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Crohn’s disease and ulcerative colitis are heterogeneous chronic inflammatory bowel diseases that result from an interplay between genetic susceptibility, microbiota in the gut and unknown environmental factors. Uncontrolled activation of the mucosal immune system results in chronic bowel damage which is not compensated for by restitution phenomena. Standard treatment involves immunosuppression with corticosteroids, azathioprine, methotrexate and antibiotics. The development of anti-TNF antibodies has opened the field for treatment with new classes of therapies based on new pathogenic insights and new technology. These therapies are broadly called biological therapies. Based on current knowledge biological therapies should aim at stimulating innate immunity or inhibit the inappropriate adaptive immune response. The anti-TNF antibodies are now part of our standard treatment. New monoclonals are being developed and also active immunisation against TNF is being explored.

Novel therapeutic compounds can be devided in to four broad categories including cytokine and anti-cytokine therapies, T-cell blocking agents, anti-adhesion molecules and growth factors. Besides TNF the main targets investigated for biological therapies currently are interleukin-12/23 p40 with the anti-p40 monoclonal antibodies CNTO 1275 (Stelara) and interference with lymphocyte migration to the inflamed intestine with selective anti-migration strategies. The most promising drug is vedolizumab, a monoclonal antibody to $\alpha_4\beta_7$ although other modalities blocking the $\alpha_4\beta_7$/ MadCam-1 interaction are being developed.

Growth factors are used to restore defects in the intestinal innate immune barrier as a whole including also the first line of defence by granulocytes. There was great interest in sargramostim, a granulocyte-macrophage colony-stimulating factor (GM-CSF) targeting neutrophils, monocytes and epithelial cells and improving leucocyte functions. Although the first results were encouraging a placebo controlled study failed to show significant results. Up to present barrier or innate immunity enhancing agents have largely failed. Another evolution in biologic IBD therapy is the development of small molecules stimulating innate immunity or interfering with the adaptive immune response. None of these have reached the clinic yet and this approach involves great challenges. Finally maybe the most attractive approach is the use of pre-and probiotics for the treatment of IBD but here also we are far from having found the exact place for these therapies in the clinic.

With all these approaches safety monitoring is of course of primary importance and unexpected toxicities have arisen and sometimes limit the use of a particular therapeutic approach.

Reference:

How genetics insights may transform clinical medicine

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Heritable components have been suggested long before confirming molecular discoveries were made by the observations of clustering of inflammatory bowel disease in large families and an increased concordance between monozygotic twins. Analysis of heritability suggested that IBD represents a “complex disease” and may involve a large number of interacting disease genes.

Crohn disease has since become a paradigm example for the successful molecular exploration of a polygenic etiology. In 2001 three coding variations in the NOD2 gene were identified that are highly associated with development of the disease. All variants affect a part of the gene that codes for the leucin rich part of the protein that appears to be involved in bacteria induced activation of NFkB in macrophages and epithelial cells. A particular subphenotype with localization of the disease in the ileocecal region is highly associated with the variants in the NOD2 gene.

Variants in the NOD2 gene by far not explain the genetic risk for Crohn disease. With the advent of high-density, genome wide association studies enormous progress has been made to discover the remaining disease genes. More than 100 disease genes have been identified until today, which however still do not fully explain the total genetic risk. In addition to innate immune barrier genes, cytokine response genes (e.g. IL-23R, IL12B, STAT3) and autophagy related genes (e.g. ATG16L1, IRGM) have been identified.

In ulcerative colitis GWAS studies are lagging behind the progress in Crohn disease. The first GWAS studies pointed among several cytokine and macrophage function related genes point to a locus in the 3’ end of the IL10 gene. In this regard the IL-10 knockout mouse becomes interesting again that in its phenotype is closer to ulcerative colitis than Crohn disease. Now more than 50 disease genes are known through large meta-analyses similar to the ones conducted in Crohn disease.

The further genetic exploration of Crohn disease and ulcerative colitis will result in molecular risk maps that are presently completed with amazing speed. Most interestingly, parallel GWAS in psoriasis, atopic dermatitis and other inflammatory diseases shows an unexpected overlap in identified disease genes and regions between the different types of inflammatory barrier diseases. The creation of medical systems biology of disease will lead to new models and eventually new therapies suitable only to subgroups of patients. It is anticipated that understanding mechanisms of manifestation will allow a better prediction of individual risks and may open the way to targeted preventive interventions.

However, before a comprehensive view of the genetic risk map is reached further etiologic discoveries remain interesting but are not yet helpful new tools for the clinician. In selected individuals, however, genetic exploration including full genome sequencing can be used to aid the choice of alternative, probatory therapies after the standard repertoire has been exhausted.
Interleukin-1β and the treatment of auto-inflammatory diseases

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Chronic inflammatory diseases fall into two categories: either “autoimmune” or “auto-inflammatory”. Although nearly all autoimmune diseases have an inflammatory component, as in rheumatoid arthritis, in autoimmune diseases, the primary defect is the auto-reactive T-cell, which is dysfunctional. The cytokines to block are TNFα, IFNγ and similar Th1 cytokines as well as IL-12/23 and IL-17. The “Auto-inflammatory Diseases” encompass several local and systemic diseases due to monocyte dysfunction, each responsive to blocking interleukin-beta (IL-1β) (1). “Auto-inflammatory Diseases” hereditary periodic fever syndromes. The best example is Familial Mediterranean Fever since the manifestations of this disease include fever, inflamed serosal and synovial tissues, biochemical markers of systemic inflammation and hematological responses of leukocytosis. Some of the syndromes have mutations in the protein “cryopyrin” and are called “cryopyrinopathies”; the same syndromes are also termed “Cryopyrin-Associated Periodic Syndromes” (CAPS); however, identical biochemical, hematological and clinical disease is also observed in patients without mutations. But auto-inflammatory diseases are also common diseases such as type 2 diabetes and gout and new data suggest heart failure. The co-cytokine in auto-inflammatory diseases is IL-6 as this cytokine drive CRP, thrombocytosis and polyclonal B-cell activation. What is the common link? The common link is loss of the tight control over the processing and secretion of IL-1β from the activated monocyte. The processing and secretion of IL-1β is a function of the IL-1β/caspase-1 “inflammasome”, a complex of intracellular proteins that results in the secretion of IL-1β. IL-1 blockade or neutralization results in a rapid and sustained reduction is disease severity; the responses are highly consistent when blocking the activity of IL-1β in these diseases. The measurement of IL-6 in the circulation is the preferred method for assessing the severity of the inflammation in auto-inflammatory diseases and the fall in serum IL-6 reveals the effectiveness of anti-IL-1β treatment. The auto-inflammatory diseases are examples in cytokine biology that reveal a causative role of a specific cytokine in a disease process. In addition to treating CAPS with anti-IL-1β, several trials of anti-IL-1β are currently underway including type 1 and 2 diabetes (2), post-myocardial infarction heart failure (3), osteoarthritis, gout (4) and smoldering myeloma (5, 6). CANTOS, the largest trial ever in anti-cytokine therapeutics has started with enrollment of 17,200 patients (7). The hypothesis being tested is whether an antibody to IL-1β will reduce cardiovascular events in a high risk type 2 diabetes cohort with a CRP > 2.0 mg/L on standard of therapy.
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POSTER ABSTRACTS

Poster Numbers 1 – 37

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Endoplasmic reticulum stress-induced enteritis in XBP1-deficient mice is dependent on NFκB signaling

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Background: The IRE1/XBP1 pathway allows to cope with endoplasmic reticulum (ER) stress. Deletion of Xbp1 in intestinal epithelial cells (IECs) results in murine enteritis, and XBP1 is a genetic risk factor for inflammatory bowel disease. Xbp1-/- epithelia deplete Paneth cells and exhibit a pro-inflammatory phenotype via JNK. We hypothesized that hypomorphic XBP1 might overactivate NFκB via IRE1α.

Methods: The IEC line MODE-K was silenced for XBP1 or additionally for IRE1α and stimulated with TNFα. Xbp1flox/floxVillin-Cre-ERT2 mice were treated with the IKK2 specific inhibitor BAY11-7082. Activation status of the NFκB pathway was assessed on protein and mRNA levels.

Results: TNFα-stimulated XBP1-silenced MODE-Ks exhibited increased phosphorylation of IKKs, IkBα, and nuclear NFκB p65, along with increased NFκB DNA binding activity and IkBα mRNA expression, which upon co-silencing for IRE1α was restored to normal. Treatment of Xbp1flox/flox Villin-Cre-ERT2 mice with BAY11-7082 reduced the severity of enteritis to 42.6% (p < 0.01) and hyperproliferation (BrdU) of the epithelium, increased apoptosis (Tunel) and loss of Paneth cells (Lysozyme) could be prevented.

Conclusions: Unresolved ER stress in IECs due to XBP1 hypofunction overactivates the NFκB pathway, secondary to hyperactivated IRE1α. NFκB overactivation plays a central role in enteritis development and can be prevented by IKK2 blockade.

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Endoplasmic reticulum stress-induced enteritis in XBP1-deficient mice is dependent on TNF receptor signaling


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Background: Deletion of Xbp1 in the intestinal epithelium of mice results in small intestinal enteritis. XBP1 is a downstream target of IRE1α and plays a substantial role in the unfolded protein response (UPR) from the endoplasmatic reticulum. Additionally, XBP1 represents a risk factor for both forms of human IBD, Crohn's disease and ulcerative colitis. TNF receptor signaling modulates the human disease and further studies have shown an interaction of TNFrsfa with IRE1α at the ER membrane leading to JNK activation. We hypothesize that the TNF receptors regulate the ER-stress driven pro-inflammatory signaling from the ER membrane through IRE1α and therefore are important for the perpetuation of enteritis.

Methods: XBP1flox/flox Villin-Cre was crossed with TNFrsf1a and TNFrsf1b knockout mice to generate double and triple “knock-out” mice. Histopathology (H.E.) was scored with a scoring system assessing the severity of enteritis. Additionally Paneth cell depletion was measured based on H.E. slides and proliferation of the epithelium was analyzed after 24h of BrdU administration.

Results: Interestingly, XBP1ΔIEC/ΔIEC TNFrsf1a-/- presented a marked reduction of the severity of enteritis from 6.3 to 1.4 [score/cm], a significantly increased hyperproliferation and a loss of Paneth cells as previously described. XBP1ΔIEC/ΔIEC TNFrsf1a-/- and TNFrsf1b-/- mice exhibited no significant reduction of severity of enteritis. Deletion of both TNF receptors could neither prevent Paneth cell depletion nor increased epithelial proliferation.

Conclusions: First, TNFrsfa contributes to intestinal inflammation of ER-stress induced enteritis, most likely via increased recruitment of the TNF receptor to IRE1α. This effect remains unaffected when both TNF receptors are dysfunctional. Second, TNF receptor signaling does not influence the depletion of Paneth cells in our model. Third, ER-stress driven hyperproliferation is influenced by TNF receptor signaling and is uncoupled from inflammatory processes.
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Analysis of STAT3c-mediated chronic colitis

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Introduction: The inflamed intestine is a site of extensive interplay between innate and adaptive immune cells. Crohn’s disease and ulcerative colitis have been traditionally associated with a Th1 or Th2 cytokine profile, respectively. More recently, this view has been challenged by evidence on Th17 cells which seem to play an important role in the pathogenesis of inflammatory bowel diseases. Stat3 is one of the transcription factors mandatory for the development of Th17 cells.

Methods: We have addressed the role of Stat3 in a T-cell dependent model of chronic colitis by transfer of cells with constitutive active Stat3 (Stat3c) in comparison to Stat3 deficient or wildtype control cells. The influence of Stat3 activation on colitis development was studied longitudinally by mini-endoscopy and by transcriptional and translational analysis of gut residing cells including various immune cells.

Results: Whereas transfer of CD4⁺CD25⁻ wildtype cells caused moderate chronic colitis, mice receiving Stat3 deficient T-cells were completely protected. Strikingly, transfer of Stat3c cells resulted in a dramatic aggravation and much earlier onset of chronic colitis suggesting a direct link between the level of Stat3 activation in T-cells and colitogenicity. Further analysis revealed differential expression of Stat3c-mediated effector molecules which could influence innate immunity, e.g. we detected high levels of Th-17 related cytokines including GM CSF, IL17a, IL17f and IL22.

Discussion/Conclusion: Thus, our data provide further evidence for a key role of STAT3 activation in T cells in the pathogenesis of chronic colitis and interference with its downstream targets might offer a therapeutic option to inhibit proinflammatory crosstalk.
Lack of Atg7 affects Paneth cell granule formation but does not compromise immune homeostasis in the gut

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Introduction: Genetic polymorphisms of autophagy related genes have been associated with an increased risk to develop inflammatory bowel disease (IBD). Autophagy is an elementary process participating in several cellular events such as cellular clearance and non-apoptotic programmed cell death. Furthermore, autophagy may be involved in intestinal immune homeostasis due to its participation in the digestion of intracellular pathogens and in antigen presentation.

Methods: In the present study, the role of autophagy in the intestinal epithelial layer was investigated. The intestinal epithelium is essential to maintain gut homeostasis and defects within this barrier have been associated with the pathogenesis of IBD. Therefore, mice with intestinal epithelial deletion of Atg7 were generated and investigated in different mouse models.

Results: Knockout mice showed reduced size of granules and decreased levels of lysozyme in Paneth cells. However, the alterations in Paneth cell granules did not affect gut immune homeostasis. No changes were observed in gut structure or immune cell infiltration. Moreover, no effect of ATG7 deficiency was observed in experimentally induced colitis models.

Discussion/Conclusion: In conclusion, these data demonstrate that Atg7 deficiency in IECs affects Paneth cell biology without affecting gut homeostasis.
Terminal restriction fragment length polymorphism analysis of the diversity of fecal microbiota in patients with inflammatory bowel disease

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Introduction: To perform terminal restriction fragment length polymorphism (T-RFLP) analyses of fecal microbiota in inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) patients and to investigate the potential alterations in fecal bacterial communities.

Methods: A total of 104 patients with ulcerative colitis (UC, n = 54), Crohn’s disease (CD, n = 24) and IBS (n = 26) and 37 healthy subjects (HS) were enrolled. DNA was extracted from their stool samples, and the 16S rRNA genes were amplified by PCR. The PCR products were then digested with *Mspl and HinP1* restriction enzymes, and the length of the T-RF was determined. The sizes of T-RFs were rounded to the nearest number between samples to produce operational taxonomic units (OTUs). A one-way analysis of similarity (ANOSIM) was used to compare the microbial communities. Analysis of similarity percentages (SIMPER) was done to determine the overall average dissimilarity and the OTUs significantly contributing the dissimilarity in microbial community compositions among the groups. The bacterial species of the significantly different OTUs were predicted from the database we developed (http://microbiology.cau.ac.kr) based on the silico PCR amplification and restriction of 16S rRNA sequences.

Results: The composition of the fecal bacterial community was significantly different between the patient groups and the HS (P < 0.05). It was also significantly different among all disease groups except between UC and IBS. Dissimilarities between the patient groups and the HS were as following: UC, 62.7%; CD, 68.4%; IBS, 61.5%. In UC patients, *streptococcus, Megasphaera* and *Bacteroides* species were significantly abundant, and *Bacillus, Megasphaera elsdenii, Selenomonas ruminatum, Rumino-coccus, Clostridium, Selenomonas, Enterobacter* species were deficient compared to the HS. In CD patients, *Megasphera, Streptomyces* and *Sphingomonas* were significantly abundant, and *Bacillus, Megasphaera elsdenii, Selenomonas ruminatum, Lactobacillus, Ruminococcus, Clostridium, Bacteroides* and *Sphingobacterium* were deficient. In IBS patients, *Actinobacillus* species were abundant and other bacteria were also detected abundantly similar to those of UC patients. *Bacillus, Clostridium, Megasphaera elsdenii* and *Selenomonas rumintitum* species were commonly deficient in all groups of patients.

Discussion/Conclusion: The composition of fecal microbiota in patients with UC, CD, or IBS significantly differs from that of HS, and also different from each other patient group except between UC and IBS.
End-stage methotrexate-associated chronic liver disease in the United States: Interaction of drug, host and environmental factors

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Introduction: Methotrexate (MTX) is an effective and widely used immuno-suppressant; however, long-term therapy has been associated with steatosis, progressive hepatic fibrosis and cirrhosis. Given the similarity of the histological features of methotrexate-associated chronic liver disease (MTX-CLD), non-alcoholic steatohepatitis (NASH) and alcoholic liver disease (ALD), we hypothesized that these diseases may share a common pathogenesis.

Methods: We analysed the diagnostic records of all individuals who have been listed for liver transplantation in the United States and reported to the Organ Procurement and Transplantation Network (OPTN) during the period 1st October 1987–22nd May 2009 to identify those whose liver disease was deemed to have been, wholly or partly, caused by methotrexate (MTX-CLD). We compared the demographic, clinical and laboratory characteristics of adult individuals with MTX-CLD with those listed for ALD and NASH.

Results: Among 148,639 unique listings for liver transplantation, we identified 105 individuals with MTX-CLD, and these were compared with individuals listed for ALD (n = 17,592) and NASH (n = 3,259). Concurrent liver disease among individuals with MTX-CLD included ALD in 4.8%, NASH in 7.7%, hepatocellular carcinoma in 2.9%, hepatitis C infection in 1% and other drug-induced liver disease in 1%. Compared to the ALD group, those with MTX-CLD were older (median age 57 vs. 51 years, p < 0.0001), more likely to be Caucasian (91.4% vs. 80.9%, p < 0.007), female (46.2% vs. 19.2%, p < 0.001) and diabetic (36.8% vs. 18.3%, p < 0.001), and had a higher body mass index (median 28.2 vs. 27.2 kg/m\textsuperscript{2}, p < 0.03) but a lower median MELD score (14.5 vs. 16, p < 0.007). In contrast, compared to individuals with NASH, those with MTX-CLD were less likely to be diabetic (36.8% vs. 47.7%, p < 0.05), had a lower median body mass index (28.2 vs. 32.1 kg/m\textsuperscript{2}, p < 0.0001) but a similar age, gender, ethnicity and MELD distribution. Multivariable analyses yielded similar results. The prevalence of a history of hypertension and vascular disease did not differ among the three groups, nor did their complications (ascites, encephalopathy, spontaneous bacterial peritonitis) profile.

Discussion/Conclusion: This is the largest analysis of end-stage MTX-CLD reported to date, demonstrating that it is a rare form of cirrhosis that has a distinct risk factor profile from that of ALD and, to a lesser extent, NASH. The severity of MTX hepatotoxicity may be potentiated by host (ethnicity) and environmental (diabetes, obesity) factors, ultimately leading to decompensated disease. A common pathogenic process may underlie MTX-CLD, ALD and NASH.
Dendritic cell subsets and serum interleukin-12 in schistosomal hepatic fibrosis: Relation to severity of liver disease and renal injury

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Introduction: Chronic infection with schistosomiasis is associated with down-regulation of T-cell immune responses that require activation by innate immune cells like dendritic cells (DCs). The present work was designed to study the DC subsets (myeloid and plasmacytoid) in peripheral blood and serum levels of interleukin (IL)-12 in patients with schistosomal hepatic fibrosis (SHF) in relation to severity of liver disease and renal injury.

Methods: Forty five patients with SHF and 15 healthy subjects were included in the study. The severity of liver disease was assessed using Child-Pugh classification and the Model of End-Stage Liver Disease (MELD) score. Renal injury was assessed by measuring urinary albumin excretory rate (UAER). The percentages of CD11c⁺ myeloid and CD123⁺ plasmacytoid DC subsets in peripheral blood were identified using 3-color flow cytometry. Quantitative determination of IL-12p70 heterodimer in serum was performed using enzyme-linked immunosorbent assay. Renal biopsies from patients with macroalbuminuria were examined by immunohistochemical technique for DCs and angiogenesis using antibodies against OX62 and factor VIII-related antigen respectively.

Results: The percentages of circulating CD11c⁺ and CD123⁺ DC cells, CD11c⁺DC/CD123⁺DC ratio and serum IL-12 levels showed significant decreases in patients with SHF compared with healthy subjects (P < 0.01). Renal tissues from patients with macroalbuminuria showed significant increases in the number of DCs and angiogenesis compared with normal renal tissues (P < 0.01). The percentages of circulating DC subsets and serum IL-12 levels were inversely correlated with Child-Pugh score, Meld score, number of renal DCs, renal angiogenesis and UAER and were positively correlated with creatinine clearance. The number of renal DCs showed positive correlations with UAER and renal angiogenesis and negative correlation with creatinine clearance (P < 0.05).

Discussion/Conclusion: DCs and IL12 seems to play a role in the progression of liver disease and renal injury in SHF. DC-based vaccines may provide a potential new goal for immunotherapy in SHF.
Peripheral blood and intrahepatic natural killer cells and activated hepatic stellate cells in chronic hepatitis C: Relation to disease activity and hepatic fibrosis

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Introduction: Natural killer (NK) cells, one of the components of the innate immune system, play an important role in the host defence against viruses and also have anti-fibrotic activity. The present work was designed to study peripheral blood and intrahepatic NK cells in patients with chronic hepatitis C (CHC) in relation to disease activity and severity of hepatic fibrosis.

Methods: Fifteen patients with treatment-naive CHC and 15 healthy subjects were included in the study. The NK and NKT cells in fresh whole blood samples were identified using two-color flow cytometry as CD3⁻CD56⁺ and CD3⁺CD56⁺ cells respectively. Liver biopsies from CHC patients were examined to assess histological activity grade and fibrosis stage according to METAVIR scoring system and for the grade of steatosis. Immunohistochemical staining was done using antibodies against CD56 and smooth muscle actin (SMA) for detection of NK cells and activated hepatic stellate cells (HSCs) respectively.

Results: The percentages of CD3⁻CD56⁺ NK cells and CD3⁺CD56⁺ NKT cells in peripheral blood showed significant decreases in patients with CHC compared with healthy subjects (P < 0.01) and were positively correlated with the intensity of intrahepatic NK cells (P = 0.001). The CD56⁺ NK cell infiltrate was found to be absent or minimal in about 70% of the liver biopsies of patients with CHC. The percentages of peripheral blood NK cells and NKT cells and the intensity of intrahepatic NK cells showed significant inverse relationships with patient’s complaint of chronic fatigue, serum HCV RNA levels, steatosis grade, METAVIR fibrosis stage and intensity of activated HSCs (P < 0.05).

Discussion/Conclusion: The deficiency of NK cells may provide a mechanism for immune suppression in patients with CHC resulting in viral persistence, disease chronicity and progression of hepatic fibrosis. Restoration of the NK cell population may be a therapeutic potential for resolution of HCV infection and modulation of hepatofibrogenesis.
Effects of budesonide-UDCA combined therapy on histological evolution of primary biliary cirrhosis

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Introduction: Aim was to evaluate the efficacy and safety of two years combined therapy (UDCA plus budesonide) and compared with UDCA alone therapy, in patients with primary biliary cirrhosis (PBC).

Methods: We studied 33 patients with PBC, at stages I to III. A comparative study was performed on two groups: A group composed 20 patients which received UDCA (10–15 mg/kg/day) and B group (13 patients) treated with UDCA and budesonide 9 mg daily divided in 3 doses). We evaluated liver histology, serum levels of amino-transferase, Bb, AP at 6, 12 and 24 months.

Results: In A group, clinical symptoms significant improved in 25% of cases after 6 months, in 60.7% after 12 months and 89.28% after 24 months. The mean value of serum bilirubin concentration was reduced from 6.7 ± 2.5 mg%, at baseline, to 2.8 ± 1.3 mg% at 6 months and to 1.7 ± 0.7 mg% at 12 months. Aminotransferase values were reduced more quickly comparative with bilirubin and AP levels: with 44.6% at 6 months and 63.2% at 12 months. In B group, aminotransferase values reduced more slowly, but significant decrease AP activity after one year (p = 0.001). Inflammatory activity was significantly reduced in the combined therapy (6 cases, 46.15%) and in 4 cases (20%) with mono-therapy. Fibrosis decreased in group B in 5 cases, but in A group only in one case. After 24 months, histological stage of disease improved only in B group (3 cases). In A group, we observed side-effect at one patient (diarrhea) and in B group 2 patients presented hyperglycemia, 2 mild hirsutism and 4 osteoporosis. Most of the side effects appeared in patients with stage III PBC and only in two patients we reduced the budesonide dose.

Discussion/Conclusion: UDCA combined with budesonide improved liver histology and liver enzymes, where as the effect of UDCA mono-therapy was mainly on liver function tests.
Budesonide versus prednisone in treatment of autoimmune hepatitis

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Introduction: The aim of this study was the assessment the efficacy and safety of budesonide and azathioprine combined therapy versus prednisone in association with azathioprine in patients with autoimmune hepatitis (AIH).

Methods: We studied 42 patients (28 females/14 males, mean ages 43.2 years) with AIH. The diagnosis of AIH was based on international criteria, including biochemical tests, autoantibodies and liver tissue morphology. A comparative study was performed on two groups of patients: A group composed of 25 patients who received a combined therapy with prednisone (40 mg/day and tapered to 10 mg/day) and azathioprine (1–2 mg/kg/day) and B group treated with Budenofalk® (3 mg, oral doses three times daily) in association with azathioprine (1–2 mg/kg/day).

Results: At 6 months, complete biochemical remission occurred in 9 cases (36%) of the A group and in 11 cases (64.7%) in B group. In A group the side effects were: mild anemia (4 cases), osteoporosis (5 cases), severe leukopenia (2 cases), steroid diabetes (2 cases) and Cushing’s syndrome (3 cases). Multiple side effects were observed in 6 cases (24%). Comparative, the rate of side effects in B group was significantly reduced (27.77%) and 15 patients (83.3%) did not develop steroid-specific side effects. After 6 months, disappearance of clinical symptoms, normal liver biochemistry and histological remission was observed in 18 cases: 7 patients in A group and 11 in B group. In A group, the results of therapy after 6 months were: complete remission in 7 cases (28%), partial response in 12 cases (48%) and treatment failure in 6 cases (24%). In B group the efficacy of therapy was significantly high: partial response in 4 cases (23.5%) and treatment failure only in 2 cases (11.75%).

Discussion/Conclusion: The combined therapy budesonide and azathioprine, induces and maintains remission in patients with AIH and determined low rate of steroid specific side effects. In association with azathioprine, budesonide is more effectively than prednisone.
Th2 associated transcription factor NFATc3 protects mice in experimental colitis-model

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Introduction: NFATc3 (nuclear factor of activated T-cells), belongs to a transcription factor family and controls the activation of Th2-lymphocytes. Due to the production of inflammatory cytokine IL-4, NFATc3 can regulate inflammatory processes in IBD. Furthermore the NFATc3 gene is involved in the proliferation of cells and in apoptotic processes. Based on these facts we examined the role of the transcription factor NFATc3 in an experimental oxazolone-colitis model.

Methods: NFATc3ko mice were treated with oxazolone to induce colitis. Miniendoscopic analysis has been done to monitor the manifestation of the colitis. The colon was isolated and histological sections were taken for immunohistofluorescent staining.

Results: The NFATc3ko mice showed in contrast to the wildtype a protection of inflammation in the oxazolone-colitis model. This is evident in endoscopic analysis as well as in histological sections. To find an explanation for the protection of the KO mice Caspase-3 staining and TUNEL reaction were done. The KO mice had more apoptotic cells in the TUNEL reaction, but less pro-apoptotic cells. Additionally, there has been found in sections of the colon more FoxP3 positive cells in the NFATc3ko mice suggesting a role in cell regulation.

Conclusion: The transcription factor NFATc3 plays a pivotal role in colitis as the NFATc3ko mice were protected in the experimental model. This can be based on the facts of increased apoptotic cells and a high expression of FoxP3 positive cells in the lamina propria.
Giardiasis in irritable bowel syndrome suspected patients – An underestimated disease in adults?

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Introduction: Giardiasis is a diarrheal infection of the small intestine caused by a protozoa called Giardia lamblia. Irritable bowel syndrome (IBS) is a very frequent, difficult to treat disease with many recurrences. Both of them are widespread in adults and sometimes share similar symptoms.

Methods: 250 consecutive adults examined for recurrent symptoms suggestive for IBS performed a fecal test for the Giardia antigen (ELISA). The cohort was subdivided in 3 groups: with predominant diarrhea (IBS-D), with predominant constipation (IBS-C) and with predominant pain and/or dyspepsia (IBS-P). All the patients were explored by colonoscopy in order to exclude an organic disease of the colon. The positive patients were treated with Metronidazole (750–1000 mg/day 5 days) and reevaluated for the symptoms of IBS 3 months later.

Results: The mean age of the cohort was 41 years (19–75). Overall the positivity for the Giardia antigen was 43.2% (108/250). In the different subgroups the results were: 83.5% (66/79) of IBS-D group were positive for Giardia with 56% of them (37 patients) being asymptomatic 3 months after the eradication with metronidazole; 22.9% (22/96) of the IBS-C group were Giardia antigen positive with 18.1% (4 patients) being asymptomatic 3 months after the eradication treatment; 26.6% (20/75) of the IBS-P group had a positive ELISA test for Giardia with 40% (8 patients) being asymptomatic 3 months after the eradication treatment.

Discussion/Conclusion: Giardiosis is a common disease which may simulate an IBS and it should be eliminated with a fecal test before establishing the diagnosis of IBS, especially in patients with diarrhea predominant form.
Caspase-8 regulates TNF-α-induced epithelial necroptosis and terminal ileitis

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Introduction: Intestinal epithelial cell death is a hallmark of intestinal inflammation and has been discussed as a pathogenic mechanism driving Crohn's disease in humans. However, the regulation of epithelial cell death and its role in intestinal homeostasis remains poorly understood.

Methods: Conditional knock out mice, human biopsies, intestinal organoids

Results: High resolution endoscopy showed spontaneous inflammatory lesions in the terminal ileum of Casp8ΔIEC mice. Additionally Casp8ΔIEC mice lacked Paneth cells suggesting dysregulated anti-microbial immune functions of the intestinal epithelium, resulting in the attachment of bacteria to the epithelial cell surface. In striking contrast to the role of caspase-8 in apoptosis, Casp8ΔIEC mice showed increased cell death especially in the Paneth cell area at the base of small intestinal crypts. Dying crypt cells usually lacked typical apoptotic body formation, suggesting necrotic rather than apoptotic cell death. Additionally electron microscopy of the crypt area demonstrated cells with typical features of necrosis including mitochondrial swelling, extensive vacuole formation and intact nuclei. Epithelial cell death induced by TNF-α, was associated with increased expression of receptor interacting protein 3 (RIP3) in Casp8ΔIEC mice and was inhibited upon blockade of necroptosis. Finally, we translated the murin model to human disease. We identified high levels of RIP3 in human Paneth cells and increased necroptosis in the terminal ileum of patients with Crohn's disease, suggesting a potential role of necroptosis in the pathogenesis of this disease.

Discussion/Conclusion: Taken together, these data demonstrate a critical function of caspase-8 in regulating intestinal homeostasis and in protecting IEC from TNF-α induced Rip-mediated necroptotic cell death.
A polymorphism in melanoma differentiation-associated gene 5 is associated with spontaneous clearance of hepatitis C virus

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Introduction: 170 million people worldwide are infected with hepatitis C virus (HCV). In about 80% the infection persists and 30% of these patients develop chronic liver disease leading to cirrhosis and hepatocellular carcinoma. However, 15–20% of newly infected individuals are able to clear the infection spontaneously. Polymorphisms in genes involved in viral defence are thought to be an important reason for interindividual differences in HCV infection outcome. Rig-I-like helicases trigger innate immune responses after infection with RNA viruses including HCV.

Methods: We have, therefore, analysed 14 non-synonymous single-nucleotide polymorphisms (SNPs) in the Rig-I-like helicase-pathway-genes DDX58 (coding for Rig-I), IFIH1 (Mda-5), DHX58 (Lgp2) and MAVS (also called IPS-1, VISA or Cardif) in European patients who spontaneously cleared the virus (n = 285) or had persistent infection (n = 509).

Results: We show that the minor T allele of non-synonymous SNP in anti-viral helicase Mda-5 has a significantly higher prevalence in individuals that spontaneously resolved HCV infection as compared to patients that remained chronically infected. Patients carrying the TT genotype have a two-fold increased chance to clear HCV infections spontaneously (OR = 0.479; P = 0.002). In addition, we find an haplotype consisting of the aforementioned minor T allele and the major allele at a second non-synonymous SNP in MDA-5 that leads to an overactive MDA-5 protein and is highly associated with spontaneous clearance of HCV (OR = 24.21 (95% CI: 5.67–103.35); P = < 1x 10-6); Furthermore, we find evidence that a SNP in the gene coding for Rig-I is associated with a lower incidence of HCV infection independently of the course of the disease.
Discussion/Conclusion: This is the first study that provides evidence for the involvement of RIG-I-like helicases in incidence and clearance of HCV infection based on epidemiological data.
COX-2 immunoexpression in ulcerative colitis and ulcerative colitis-associated neoplasia

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Introduction: Because longstanding ulcerative colitis (LUC) is associated with increased risk of developing colonic cancer through a dysplasia-carcinoma sequence and dysplasia usually precedes colorectal carcinoma it is very important to identify patients at risk, by obtaining clear, earlier evidence of dysplastic mucosal changes.

Aims: The aim of our paper was to evaluate COX-2 immunoexpression in longstanding UC and UC-associated colorectal carcinoma and to correlate it with clinicopathological parameters.

Methods: We studied 33 patients with longstanding UC and 10 healthy subjects. During colonoscopy, multiple biopsy samples were taken at 10 cm intervals throughout the colorectum for histological and COX-2 immunohistochemical assessment. Patient characteristics (gender, age, disease extension, clinical activity) were evaluated. Immunohistochemistry for COX-2 was carried out using the labeled streptavidin-biotin-peroxidase complex system. The degree of staining was assessed by two pathologists independently and graded semiquantitatively using criteria previously described.

Results: The correlation between COX-2 immunoreactivity and the clinicopathological parameters analyzed showed no significant differences. COX-2 immunohistochemistry showed moderate to marked diffuse cytoplasmic overexpression in all of the biopsies with dysplasia, regardless of grade. The expression of COX-2 in dysplastic lesions (LGD and HGD) was present in most (> 90%) of the cells. Carcinoma showed marked overexpression of COX-2. The distribution within colonic crypts of COX-2 staining was variable, and revealed no consistent localization to the crypt base, mid-crypt, or surface epithelium.

Discussion/Conclusion: COX-2 showed low immunoexpression in normal colon and higher expression in UC associated colorectal carcinoma. COX-2 may probably play a role in the pathophysiologic processes of UC and in the development of neoplasia. Treatment with selective COX-2 inhibitors might be an additional option for therapy.
Assessment of inflammatory response in patients with alcoholic liver disease

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Introduction: Platelets (PLT), together with recently discovered lymphocytes T helper 17 (Th17) and regulatory T cells (Treg), are critically linked to inflammatory innate immune response. While PLT and Th17 exert pro-inflammatory effects, Treg are potent immune suppressors, but they may reciprocally regulate mutual function. The aim of our study was to clarify how PLT, Th17 and Treg participate in inflammatory response in the course of alcoholic liver disease (ALD).

Methods: We studied the frequencies of Th17 and Treg and PLT counts in peripheral blood of 47 patients (pts) with ALD in comparison to 26 healthy controls (HC). Pts were divided according to their alanine aminotransferase (ALT) [normal range (NR) 10–40 U/L] and PLT [NR 130–350 x 10³/uL] levels as follows: I- ALT ≥ twice the upper limit of NR; II- ALT < twice the upper limit of NR; III- PLT < 130 x 10³/uL; IV- PLT ≥ 130 x 10³/uL. Flow cytometric analysis FACS Calibur with CellQuest software was used to identify T cell phenotype. CD3⁺CD4⁺IL17⁺ cells were considered Th17 and CD4⁺CD25⁺FOXP3⁺ Treg. They were expressed as the percentage of all CD3⁺CD4⁺ and CD4⁺CD25⁺ lymphocytes, respectively.

Results: We found the significantly higher frequency of Th17 and lower Treg in I group in comparison to II group (0.99 ± 0.38 vs. 0.74 ± 0.36, p = 0.03; 3.49 ± 1.25 vs. 4.47 ± 1.84, p = 0.08; respectively). Pts with low level of PLT (III group) exerted significantly lower frequencies of both Th17 and Treg in comparison to pts with normal PLT counts (IV group) (0.73 ± 0.34 vs. 0.99 ± 0.53, p = 0.04; 3.43 ± 1.39 vs. 4.63 ± 1.79, p = 0.01; respectively). There was no significant difference in ALT activity between III and IV group (79.41 ± 61.61 vs. 80.32 ± 95.62, p = 0.97).

Discussion/Conclusion: The percentage of Th17 was positively and Treg negatively linked to the severity of liver inflammation in pts with ALD. Positive correlation was observed between PLT and both new subsets of lymphocytes in sera of studied patients.
Gender affects immune response in patients with alcoholic liver disease

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Introduction: The mechanisms that underlie the sex difference in alcohol-induced liver damage are not completely clarified. Females appear to be more susceptible to the toxic effects of ethanol and they have a significantly higher risk of developing cirrhosis. The aim of our study was to assess the possible interaction between gender and peripheral blood T lymphocytes that regulate immune response in patients with alcoholic liver disease (ALD).

Methods: We studied the frequency of lymphocytes T helper 17 (Th17) and regulatory T cells (Treg) in peripheral blood of 42 patients (pts) with ALD in comparison to 26 healthy controls (HC). The study population consisted of 14 women and 28 men. Pts were divided according to Child-Pugh score into 3 groups: A- 6 pts; B- 20 pts; C- 16 pts. Flow cytometric analysis FACS Calibur with CellQuest software was used to identify T cell phenotype. CD3⁺CD4⁺IL17⁺ cells were considered Th17 and CD4⁺CD25⁺FOXP3⁺ Treg. They were expressed as the percentage of all CD3⁺CD4⁺ and CD4⁺CD25⁺ lymphocytes, respectively.

Results: The frequency of Treg was significantly increased in female pts with ALD in comparison with male pts and HC (4.45 ± 1.35 vs. 3.50 ± 1.34, p = 0.03; vs. 3.48 ± 1.56, p = 0.05). The highest percentage of Treg was observed in females and the lowest in males from Child C group (4.6 ± 1.46 vs. 2.59 ± 0.74, p = 0.004). Pts from Child-Pugh A group exhibited no statistical difference in the frequency of Treg in comparison to HC. We found no significant differences in the frequency of Th17 between studied groups.

Discussion/Conclusion: We observed gender-related shift in the frequency of Treg in peripheral blood of pts with ALD. The percentage of Treg in females was positively and in males negatively linked to the severity of liver damage. It is likely that immune response may influence the sex susceptibility to the toxic effects of ethanol.
A novel genetic variant within *PNPLA3-SREBP1c* pathway represents a general risk factor for hepatic fibrosis: Elastography-based study in patients with chronic liver diseases

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**Introduction:** The common p.I148M adiponutrin (*PNPLA3*) variant is a genetic risk factor for liver fibrosis. As expression of adiponutrin is induced by sterol regulatory element binding protein 1c (*SREBP1c*) here we investigate two *SREBP1c* variants (rs2297508 and rs11868035) for their involvement in hepatic fibrogenesis.

**Methods:** In 899 individuals (age 20–86 years, 548 males) with chronic liver diseases (CLD) liver status was non-invasively phenotyped by transient elastography (TE, *Fibroscan*). *SREBP1c* single nucleotide polymorphisms (SNPs) were genotyped using PCR-based assays with 5'-nuclease and fluorescence detection.

**Results:** The rs11868035 variant significantly \( P = 0.01 \) affected liver fibrosis: Median TE levels were 7.2, 6.6 and 6.0 kPa in carriers of [TT] (n = 419), [CT] (n = 380) and [CC] (n = 100) genotypes, respectively. Comparison of individuals with mild (i.e. TE < 7 kPa, n = 480) and enhanced (TE ≥ 7 kPa, n = 419) fibrosis showed association \( (P = 0.004) \) of the SNP and TE levels (OR = 1.34, 95% CI 1.10–1.63). In multivariate models, *SREBP1c, PNPLA3* variants and age increased liver fibrosis (all \( P < 0.05 \)). Carriers of both PNPLA3 risk [GG] genotype and the SREBP1c [TT] or [TC] variants (n = 54) displayed higher \( (P < 0.05) \) TE levels as compared to patients with other genotypes (median TE 7.7 vs. 6.7 kPa).

**Discussion/Conclusion:** The common *SREBP1c* polymorphism affects early stages of liver fibrosis. This underscores the pivotal role of the *SREBP1c-PNPLA3* pathway in hepatic scarring.

**Reference:**

Biliary anti-neutrophil cytoplasmic antibodies are associated with disease severity in primary sclerosing cholangitis

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Introduction: Primary sclerosing cholangitis (PSC) is an autoimmune cholestatic liver disease of unknown aetiology, characterized by progressive inflammation of the intrahepatic and extrahepatic bile ducts causing biliary strictures. Anti-neutrophil cytoplasmic antibodies (ANCA) have been found in serum of patients with PSC, but their clinical relevance remains unclear. Since inflammation of PSC takes place in the biliary epithelium we hypothesized that an autoimmune marker such as ANCA may be detected in bile, potentially providing diagnostic and prognostic information.

Methods: Bile and serum samples were prospectively collected during endoscopic retrograde cholangiopathy (ERC) in 52 patients including 36 patients with PSC, 7 patients with a cholangiocarcinoma (CC) without PSC and 8 patients with other biliary obstructive disorders. ANCA measurements were performed in both bile and serum by indirect immunofluorescence (IIF). We recorded the number of ERCs and interventions, presence of dominant strictures, cholangiographic severity of the disease (Amsterdam Score) and clinical endpoints including transplantation, CC or death.

Results: In PSC patients, ANCA were present in sera and bile of 33 (92%) and 13 (36%) patients, respectively. ANCA positivity in bile was significantly associated with the presence of dominant strictures (p = 0.03), the Amsterdam Score (p = 0.005) and number of ERC procedures (p = 0.009) and interventions performed (p = 0.01). However, the presence of ANCA in bile did not correlate with transplantation, CC or death. No association of ANCA positivity in sera was observed with the above-mentioned clinical features. In patients with other biliary diseases, 6 (23%) out of 26 patients had ANCA-positive sera, but ANCA were only detected in the bile of one patient with CC without PSC.

Conclusion: The presence of biliary ANCA positivity in patients with PSC is a novel finding. In contrast to serum ANCA, biliary ANCA correlate with the severity of bile duct strictures and subsequent numbers of ERCs and interventions. Positive ANCA status in bile may serve as a prognostic marker of disease progression and biliary complications.
Relationship between plasma concentrations of fibronectin and endotoxin antibodies in chronic hepatitis (CH) and liver cirrhosis (LC)

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The aim of the investigation was to evaluate the relationship between concentrations of plasma fibronectin (FN) containing endotoxin antibodies (EA) as a major marker of immune response to endotoxin (LPS) of gram-negative organisms of intestinal ecosystem in CH and LC.

Methods: 42 CH, 97 LC patients and 36 healthy volunteers investigated the concentration of FN and EA by ELISA method.

Results: The concentration of FN in CH averaged 240.3 ± 16.0 µg/ml, LC – 220.9 ± 11.6 µg/ml, normal – 348.9 ± 23.4 µg/ml. Significant reduction of FN in CH was observed in 41% of cases, LC – 64%. One reason for reducing of the concentration of FN may be linked with the increased consumption of opsonins in the process of binding and elimination of bacterial endotoxins from the blood stream. Increased level of the endotoxin concentration in CH in some patients with LC indirectly estimated by the content of EA. Average levels of EA in CH and LC did not differ significantly from the norm (7.7 ± 4.5 µg/ml and 9.1 ± 5.9 µg/ml vs. 8.0 ± 0.38 µg/ml), but in 51.8% CH and 46.5% LC patients EA value was significantly above normal, while 40.8% in CH and 44.2% in LC was below the norm. The negative correlation between concentrations of FN and EA (r = -0.614 and -0.424) was determined in CH and LC.

Conclusion: In CH and LC patients with the growth of the level of EA decreased the concentration of FN, which reflected the great importance of this glycoprotein in the implementation of endotoxemia.
Inflammatory responses of the intestinal epithelium

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Introduction: The intestinal epithelial cell layer is in constant contact with the gut microflora. By expressing different pattern recognition receptors, intestinal epithelial cells (IECs) are able to produce cytokines and chemokines in response to microbial stimuli, thereby interacting with immune cells. Intestinal epithelial cells undergo anoikis, a form of apoptosis, shortly after isolation and are often contaminated with intestinal lymphocytes which also produce cytokines.

Methods: Here we analyzed gene expression of primary murine intestinal epithelial organoids, reflecting the physiological structure of the gut containing all types of intestinal epithelial cells. That allows the characterization of IEC gene expression in response to microbial stimuli without contaminating transcription levels of cytokines produced by immune cells.

Results: Our data show that primary IECs respond differentially towards TLR stimuli. For example treatment with zymosan that stimulates TLR2 results in an increased expression of IL-7. Furthermore, IECs respond to TLR9 stimulation via CpC oligodeoxynucleotide with a lower IL 33 expression. Whereas primary epithelial cells responded little towards poly I:C or Pam2CSK4.

Discussion/Conclusion: For the first time we were able to analyze TLR-receptor mediated expression of pro- and anti-inflammatory cytokines in an in vitro culture of primary murine IECs. Together our data demonstrate that this primary epithelial organoid culture is an excellent method to identify genes produced by IECs in response to microbial influences on inflammation. These results are helpful to understand the mechanism underlying the defective immune response of IECs in patients suffering from IBD and will help to increase our understanding of the pathogenesis of this disease.
Interleukin-33/ST2 signalling in the pathogenesis of liver fibrosis

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Introduction: Hepatic fibrosis represents a life-threatening health problem worldwide of which no applicable therapy exists. It is accompanied by the accumulation of extracellular matrix in response to acute or chronic liver injury and may ultimately lead to cirrhosis. IL-33, a novel member of IL-1 family of cytokines signalling via its receptor (ST2), is known to be involved in several autoimmune and inflammatory disorders; however, its function in liver fibrosis still remains enigmatic.

Methods: We have analyzed IL-33 transgenic, expression vector-based overexpressing, knockout and receptor knockout mice.

Results: Different experimental fibrosis models were assessed where IL-33 knockout mice were significantly protected. In order to analyze cellular targets of the profibrotic function of IL-33, we performed bone marrow chimeric experiments, where ST2⁻/⁻ mice revealed protection against fibrosis denoting the relevance of hematopoietic cells. Gene chip analysis of liver from IL-33 overexpressing mice identified several gene candidates being differentially regulated during fibrosis, including chemokines and type 2 IL-4 receptor (IL-4R) signalling molecules. Accordingly IL-4Rα⁻ and IL-13 knockout mice were almost completely protected from IL-33 induced fibrosis. As far as analysis of immunodeficient mice (Rag1⁻/⁻) indicated to the important role of innate immune cells, further studies were accomplished that identified the individual contribution of certain innate immune cell population to development of liver fibrosis.

Discussion/Conclusion: Consequently, our findings suppose to have important implementation to impede a range of fibrosis-based diseases.
Urine proteomic analysis differentiates cholangiocarcinoma from primary sclerosing cholangitis and other benign biliary disorders


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Background: Diagnosis of cholangiocarcinoma (CC) and subsequent curative treatment often comes too late due to the lack of early symptoms and reliable tumour markers especially in patients with primary sclerosing cholangitis (PSC). A convincing PSC surveillance strategy for early CC detection is urgently required. We recently showed that bile proteomic analysis distinguishes CCs from non-malignant lesions. As a non-invasive diagnostic method to differentiate CCs from other biliary disorders, we hypothesized that urine which harbours less number of peptides than bile, might be a new target for proteomic analysis.

Methods: In this prospective study, we used capillary electrophoresis mass spectrometry (CE-MS) to establish a disease specific peptide model in a training set of patients with CC (n = 14), PSC (n = 13), and benign biliary diseases (BBD, n = 14). Peptides were characterized by sequencing and immuno-staining was performed on liver sections.

Results: The peptide model consisting of 42 peptides was evaluated on an independent set of 128 urine samples from 42 CC- (including 10 CC on top of PSC), 50 PSC- and 36 BBD-patients. This model differentiated CC from PSC and BBD with an area under the curve value of 0.87 (95% confidence interval: 0.80–0.92, p = 0.0001) resulting in a correct classification of 35 from 42 CC and 68 from 86 benign strictures (83% sensitivity, 79% specificity). Ten of 10 patients with CC on top of PSC were correctly classified. 101 healthy controls were analyzed and specificity was determined to be 86%. The majority of sequence-identified peptides are fragments of interstitial collagens with some of them also detected in blood indicating their extra-renal origin. Immuno-staining of liver sections for matrix metalloproteinase 1 (MMP-1) indicated increased activity of the interstitial collagenase in liver epithelial cells of CC patients.

Conclusion: The urinary peptide model differentiates CC from PSC and other benign biliary diseases and may become a new diagnostic non-invasive tool for PSC surveillance and CC detection.
Intra- and extracellular pre-B cell colony-enhancing factor modulates hepatic inflammation in experimental hepatitis

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Pre-B cell colony-enhancing factor (PBEF), also known as nicotinamide phosphoribosyltransferase or visfatin, is involved in metabolic, inflammatory, and malignant diseases. PBEF exerts both an extracellular cytokine-like function and an intracellular enzyme function. Experimental data suggests that blocking its enzymatic activity using a specific small-molecule inhibitor (FK866) might be beneficial in the treatment of acute experimental inflammation.

In our recent study we investigated the role of PBEF in human liver disease and experimental hepatitis. PBEF serum levels and hepatic expression were determined in patients with chronic liver diseases. Inflammatory responses were studied in lentivirally PBEF-silenced or control FL83B mouse hepatocytes. The effect of extracellular PBEF was examined in primary Kupffer cells, hepatocytes, and liver sinusoidal endothelial cells. These studies were completed by in vivo experiments using concanavalin A (ConA) and D-galactosamine/lipopolysaccharide (LPS) models of experimental hepatitis. PBEF was either overexpressed by hydrodynamic perfusion or inhibited by FK866.

Serum PBEF concentrations were significantly higher in patients with chronic liver diseases irrespective of disease stage and etiology. In vitro, PBEF-silenced mouse hepatocytes showed decreased responses after stimulation with LPS, lipoteichoic acid, and tumor necrosis factor a. In primary murine Kupffer cells, FK866 suppressed LPS-induced interleukin (IL)-6 production, whereas incubation with recombinant PBEF resulted in increased IL-6 release. Liver-targeted overexpression of PBEF rendered mice more susceptible to ConA- and D-galactosamine/LPS-induced hepatitis compared with control animals. In contrast, inhibition of PBEF using FK866 protected mice from ConA-induced liver damage and apoptosis. Administration of FK866 resulted in depletion of liver nicotinamide adenine dinucleotide1 levels and reduced proinflammatory cytokine expression. Additionally, FK866 protected mice in the D-galactosamine/LPS model of acute hepatitis.

Taken together our data suggest that PBEF plays a key role in experimental liver disease. Its specific inhibition might be considered a novel treatment option for inflammatory liver diseases.
Chronic pancreatitis with autoimmune origin in children

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Introduction: In the last decade we can observe gradual increase of autoimmune diseases. The reported paediatric experience with chronic pancreatitis (CP) is small and little is known about the role of autoimmune chronic pancreatitis (AICP).

Aim: The aim of the study was to assess the frequency of autoimmune markers in children with CP.

Methods: During 2000–2010, we hospitalized 66 children with CP (35 boys and 31 girls; age 2–18 years, mean age: 9.1 years). Clinical data were recorded and analyzed. Gammaglobulins, IgG4, autoantibodies (ANA, ASMA, AMA, APCA, LKM and AHA) were measured in all children.

Results: Autoimmune disease was present in 5 patients (7.6%): ulcerative colitis in 2 patients, PSC, dermatomiositis and panniculitis in 1 patient each. Hypergammaglobulinemia (> 16 g/l) was present in 14 cases. An increase of IgG4 level was present in 5 children. Autoantibodies were present in 38 children (57.6%). ANAs (> 1/80⁰) were present in 18 patients with CP (in 6 pts > 1/640⁰). ASMAs (> 1/80⁰) were present in 21 children. APCAs, AMAs, AHAs and LKM were absent in all patients. Combining clinical and biochemical autoimmune parameters, 41 patients (62.1%) had at least 1 autoimmune marker of the disease. In 20 patients (27.4%) with CP and autoimmune stigmata other known causative factors of CP were present. In 16 patients we found gene mutations predisposing to CP. There was no difference in the severity of the disease and clinical course between children with autoimmune stigmata and patients without autoimmune markers. 2 patients were treated with steroids with good clinical response.

Discussion/Conclusion: In children with CP, similarly to adults, there is a high frequency of clinical and biochemical markers of autoimmunity. Number of CP with autoimmune origin in children is greatly underreported.
Immunogenetic features of HCV-related fatigue

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Introduction: Fatigue is the most common complaint in chronic hepatitis C. The recent findings suggest that HCV infection of the central nervous system may be related to fatigue development. The 2',5'-oligoadenylate synthetase (OAS) system is an important component of the innate immunity pathway that responds to double-stranded RNA structure within nontranslated regions HCV RNA to induce degradation of viral RNAs. The A allele of an A/G splice-site single nucleotide polymorphism rs10774671 in the OAS1 gene encoding 2',5'-oligoadenylate synthetase has been associated with low enzyme activity. The aim was to investigate whether the OAS1 variant could influence the occurrence of fatigue in HCV infected patients.

Methods: Eighty-eight patients with chronic hepatitis C and 122 healthy controls were included in the study. Cancer-related fatigue (CRF) diagnostic criteria with slight modifications were used to define fatigue. The A/G OAS1 SNP rs10774671 genotyping was performed by PCR-RFLP.

Results: The prevalence of fatigue in HCV infected patients was 60%. There was statistically significant difference in the OAS1 gene polymorphism between HCV-infected patients with fatigue and those without fatigue ($\chi^2 = 6.93; \text{df} = 2; \text{p} = 0.031$). Patients with fatigue more frequently had the A allele at rs10774671 as compared to patients without fatigue (74% versus 56%; OR = 2.32; 95% CI = 1.16–4.65). No significant differences were observed in the distribution of allele and genotype frequencies of the OAS1 gene polymorphism between HCV infected patients and healthy control subjects.

Discussion/Conclusion: The functional polymorphism of the OAS1 gene analyzed in this study might affect fatigue development in patients with chronic HCV infection.
Expression of hepcidin and heme oxygenase-1 in chronic hepatitis

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Aim: Oxidative stress is an element of HCV-related liver injury. Iron overload in chronic hepatitis C (CHC) may contribute to liver fibrosis progression and carcinogenesis. Heme oxygenase-1 (HO1), a cytoprotective and antioxidant enzyme may be induced by iron and decrease expression of HCV proteins. We investigated the association of iron parameters with hepcidin (HAMP) and HO1 gene expression in liver tissue in chronic hepatitis.

Methods: Liver function tests, iron, ferritin concentration, transferrin saturation were assayed in 31 patients with CHC and 19 with chronic hepatitis B (CHB). Inflammation activity, fibrosis, presence of iron deposits were assessed in liver specimens in both groups. HFE gene mutations were detected using PCR-RFLP methods. Measurement of HAMP and HO1 mRNA expression was done using two-step quantitative real-time RT-PCR with normalization to two stably expressed reference genes.

Results: Hepatocyte iron deposits and more advanced liver fibrosis were found in CHC more frequently. HAMP gene expression was significantly lower and positively correlated with ALT activity, serum iron concentration, HO1 gene expression in CHC. HO1 gene expression was lower but without significance and positively correlated with grade of inflammation only in CHC. Serum iron parameters and HAMP and HO1 expression were independent on presence of HFE gene mutations. expression did not correlate with hepatocytes iron deposits in both groups.

Conclusions: HAMP and HO1 expression were not induced by hepatocytes iron deposits. HAMP and HO1 expression were lower in CHC despite of presence of active hepatitis markers possibly as a result of mechanisms specifically activated by HCV proteins.
The role of Kupffer cells in the pathogenesis of non-alcoholic steatohepatitis: Immunohistochemical and ultrastructural findings in pediatric patients

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Background: Nowadays there is a growing incidence of pediatric form non-alcoholic steatohepatitis (NASH) in developed countries. The pathogenesis of NASH, a common disease worldwide and a major cause of cryptogenic cirrhosis of the liver still remains unclear. It is a clinicopathologic condition characterized by a necroinflammatory disorder with fatty infiltration of hepatocytes and with/without fibrosis. Recently, it has been speculated that Kupffer cells/macrophages (KCs) may be involved in the disease pathogenesis, especially in reference to the experimental animal models of NASH.

Aim: The main objective of the current study was to explore the role of KCs, a type of hepatic nonparenchymal cells (NPCs), in the morphogenesis of NASH in children.

Methods: Immunohistochemical (IHC) and ultrastructural investigations were conducted on biopsy liver specimens obtained from 10 children (6 boys and 4 girls), aged 2–14 years, and with previously clinicopathologically diagnosed NASH. All liver biopsy specimens were IHC examined for Kupffer cells/macrophages (CD68) and penetrated with a transmission electron microscope (TEM). IHC staining to identify KCs was performed using monoclonal mouse antibody CD68 (Clone PG-M1, M0876, Dako, Denmark). The material for ultrastructural investigations was fixed in solution of 2.5% glutaraldehyde and 2% paraformaldehyde, routinely processed for TEM analysis and examined using an Opton EM microscope.

Results: IHC examinations for biomarker CD68 fully identified KCs within the liver bioplates. The number of CD68 Kupffer cells/macrophages in the course of NASH showed a significant increase. These cells were markedly activated and exhibited strong or very strong immunoexpression of the biomarker used. The current ultrastructural study revealed within the hepatic sinusoids the presence of numerous enlarged Kupffer cells with increased phagocytic activity, which markedly reduced the lumen of these vessels, including their blockade, and led to hepatic microvascular structure damage. Interestingly, apart from primary and secondary lysosomes and well-developed Golgi apparatus within the activated KCs, also absorbed fragments of erythrocytes, varying in size, were quite frequently seen. Therefore, we may assume that erythrophagocytosis by hepatic macrophages may be responsible for hepatic iron accumulation in this pathology. Worthy of notice is that activated Kupffer cells were seen very close to the transformed hepatic stellate cells and hepatic progenitor/oval cells. Features of intensive fibrosis were frequently observed in the vicinity of activated KCs/macrophages.
Discussion/Conclusion: Engulfment of erythrocytes by hepatic macrophages may lead to the accumulation of iron derived from hemoglobin in the liver and involvement in the pathogenesis of NASH in pediatric patients. This type of NPCs may play a key role in triggering the generation of oxidative stress. It should be assumed that iron reduction therapy may be promising in NASH patients.
Correlation of hepatic steatosis with iron overload in chronic hepatitis C

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Introduction: Hepatic steatosis and iron overload, frequently observed in chronic hepatitis C (CHC), may contribute to progression of liver injury. The analysis of correlation between liver steatosis and iron overload in Polish patients infected with hepatitis C virus (HCV) was the study aim.

Methods: 191 consecutive CHC patients (84% HCV genotype 1), mean age 44 were included to the study. The control group consisted of 172 patients with non-HCV chronic liver disease. Liver function tests, serum markers of iron metabolism, cholesterol and triglycerides were assayed. The inflammation activity, fibrosis, iron deposits and steatosis stages were assessed in liver biopsy. HFE gene polymorphism was investigated by PCR – RFLP.

Results: The hepatocytes steatosis, strongly associated with overweight and diabetes mellitus, was confirmed in 75/172 CHC patients (regardless of HCV genotype and viral load) and 79/145 non-CHC patients. The average grade of liver steatosis was higher in non-CHC patients. The iron concentration and transferrin saturation were significantly higher in CHC than non-CHC patients with liver steatosis. Moreover, hepatocytes steatosis was found significantly more frequently in CHC patients with total serum markers indicating iron accumulation than in patients with normal iron parameters (p = 0.02). HFE gene mutations were detected more frequently in all patients with hepatocytes iron deposits. No correlation of HFE gene polymorphism with liver steatosis in CHC was found.

Discussion/Conclusion: The frequency of liver steatosis in CHC and non-CHC is similar but intensity of steatosis is higher in non-CHC patients. In CHC patients biochemical markers of iron accumulation positively correlated with liver steatosis. Liver steatosis did not correlate with HCV genotype or viral load. HFE gene mutations were associated with iron overload but not with hepatocytes steatosis in non-CHC patients.
Interplay between systemic markers of inflammation and kidney function in patients with alcoholic liver disease

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Introduction: Since the well-known precipitating factors of hepatorenal syndrome include spontaneous bacterial peritonitis and superimposed infections, we hypothesize that mediators of the inflammatory response may play a role in the development of this condition. The aim of our study was to assess an interplay between serum markers of inflammation and kidney function in patients (pts) with alcoholic liver disease (ALD).

Methods: We studied markers of inflammatory response: 1) T helper 17 cells (Th17); 2) regulatory T cells (Tregs), 3) C-reactive protein (CRP) level, and 4) white blood cells count (WBC) in peripheral blood of 55 pts with ALD with impaired and normal kidney function in comparison to 21 healthy controls (HC). Pts were divided into two groups according to their serum creatinine level (CREA): I group n = 9, CREA > 133 µmol/l; II group n = 46: CREA ≤ 133 µmol/L. Flow cytometric analysis FACS Calibur with CellQuest software was used to identify T cell phenotype. CD3⁺CD4⁺IL17⁺ cells were considered Th17 and CD4⁺CD25⁺FOXP3⁺ Tregs. They were expressed as the percentage of all CD3⁺CD4⁺ and CD4⁺CD25⁺ lymphocytes, respectively.

Results: The frequency of Th17 cells was significantly increased in pts from I group in comparison with pts from II group and HC (1.37 ± 0.81 vs. 0.80 ± 0.36; p = 0.002; vs. 0.95 ± 0.39; p = 0.03). The frequency of Tregs was highest in pts from I group, but the difference was not statistically significant (4.18 ± 1.76 vs. 3.69 ± 1.37 vs. 3.72 ± 1.82 respectively; p = 0.43). There was no statistically significant difference in CRP level and WBC between both studied groups. 3 deaths occurred in I group and 3 in II group (p = 0.04).

Discussion/Conclusion: Patients with ALD and impairment of kidney function express significantly higher serum Th17 cells frequency in comparison to pts with normal kidney function and HC. Their risk of death is increased as well.
Increased apoptosis of regulatory T cells in patients with chronic inflammatory bowel disease

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Background and aims: Inappropriate immune responses contribute to the continuous stimulation of the intestinal immune system in chronic inflammatory bowel disease (IBD). Among several pathogenic factors, a numerical deficiency of regulatory T (Treg) cells has been suggested to lead to an insufficient compensation of chronically activated T lymphocytes. In this study, we investigated whether increased apoptosis contributes to Treg cell deficiency in IBD.

Methods: We analyzed apoptosis of CD4⁺Foxp3⁺ Treg cells in tissue sections of patients with active IBD by immunohistochemistry and TUNEL staining. Apoptosis of peripheral blood CD4⁺CD25⁺Foxp3⁺ Treg cells of IBD patients was analyzed by flow cytometry and annexin-V staining. In addition, we measured caspase activity in sera of patients with active IBD or IBD in remission by a luminometric caspase enzyme assay.

Results: We could demonstrate that patients with active IBD revealed increased apoptosis of local CD4⁺Foxp3⁺ Treg cells in the inflamed mucosa compared to non-inflamed control colon tissue. Moreover, in peripheral blood a reduced frequency and increased apoptosis of Treg cells were found and accompanied by elevated caspase activity in the serum in patients with active IBD.

Conclusions: These data suggest that increased apoptosis of Treg cells plays a potentially important role in the IBD pathogenesis. Measurement of Treg cell apoptosis and serum caspase activity might therefore represent promising tools for monitoring disease activity in IBD patients.
Secondary sclerosing cholangitis in critically ill patients: Clinical presentation and prognostic factors for fatal outcome

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Introduction: Secondary sclerosing cholangitis in critically ill patients (SSC-CIP) is an underdiagnosed emerging disease entity. Our aim was to characterize the clinical presentation of patients with SSC and to identify prognostic factors for fatal outcome.

Methods: Retrospective analysis for 54 patients after intensive care unit (ICU) treatment, who were diagnosed as SSC via ERCP after cardio-thoracic surgery (CTS) interventions (n = 21), sepsis (n = 13), poly-trauma (n = 11), and others (n = 9). Bile samples for microbiological analysis were obtained during ERCP.

Results: Thirty-three patients either died (n = 27) or needed liver transplantation (n = 6) were compared to surviving patients (n = 21). We identified septic complications, fever before ERCP, hemodialysis, elevated bilirubin, a high MELD Score and low albumin as risk factors. The diagnostic success rate was 30% for abdominal ultrasound, 36% for liver biopsies and 100% for ERC with only one patient who developed mild post-ERCP pancreatitis. However, the time gap between onset of severe cholestasis and ERCP diagnosis was 74 ± 68 days (range 8–305). Microbiological analysis of bile revealed colonization in all bile samples but one. An escalation of antimicrobial therapy was necessary in 28% of cases.

Conclusion: SSC in CIP is a rare but unfortunately frequently fatal disease. The diagnostic procedure of choice is ERCP, but it often comes late because other causes for cholestasis are taken into account. In presence of the summarized risk factors above, an early ERCP should be considered and bile should be obtained for microbiological analysis to adjust the antibiotic regime.
Analysing the role of mucosal mast cells inducing colitis-associated colorectal cancer

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Introduction: Colorectal cancer is one of the most malignancies. However, the molecular pathogenesis of colorectal cancer is poorly understood. In order to investigate the functional role of mast cells, which play a more prominent role in immunological processes, we used a previously established murine colon carcinoma model (DSS/Azoxymethan) with mast cell deficient mice.

Methods: Accordingly, mice were treated with AOM followed by three consecutive cycles of orally administrated dextran sulfate sodium (DSS) over a period of 7 days. To monitor tumorigenesis in mice in vivo, we used our mini-endoscopic system.

Results: By using this system together with methylene blue staining, we were able to detect aberrant crypt foci in DSS plus AOM-treated wild-type mice at early time point before macroscopically visible lesions were seen. First visible lesions associated with inflammation appeared in wt mice around day 45, which were followed by the development of more and growing tumors until day 90. In contrast, mast cell deficient mice are protected against tumor development and although they showed colitis-similar symptoms. The possibility, that mast cells play a tumor-promoting role in the development of colon tumors led us to perform a screening of the expression of involved cytokines in colons and tumors of treated mice versus untreated mice. Even in long term study, a marginal increase of the tumor prevalence concerning mast cell deficient mice could be observed.

Discussion/Conclusion: Our data contribute extensively the understanding of mast cells in colitis-associated colon cancer and encourage of rethinking the role of mast cells in colitis-associated colorectal cancer.
Interferon-λ signalling drives pathogenic immune responses during microbiota induced septic peritonitis

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Introduction: Recent studies demonstrated that IFN-λs are an important part of TLR-mediated host responses in viral infections and showed association of reduced IFN-λ production with rhinovirus-induced asthma exacerbation. However, the role of these novel cytokines in bacterial induced diseases remains poorly understood. Here we investigated the role of IFN-λ dependent immune-responses in bacterial infections.

Methods: Mice deficient in IFN-λ signal transduction or that systemically overexpress IFN-λ were analyzed in experimental models of gut microbiota induced polymicrobial sepsis and gram negative septic shock.

Results: Initially, we analyzed IFN-λ expression in mice systemically subjected to the intestinal microbiota. As a result qPCR/ELISA analysis demonstrated a strong upregulation of IFN-λ expression in serum/organs that depended on the presence of TLR4 and the IRF3 transcriptional activator. Furthermore, mice deficient in IFN-λ signaling, subjected to cecal-ligation-and-puncture (CLP) showed significantly increased survival compared to controls. Survival post CLP model was associated with highly decreased systemic spread of infection, inflammatory responses and organ injury. Similar results were obtained in a model of LPS induced septic-shock, where high apoptosis in IFN-λ receptor expressing intestinal epithelial cells was observed. In line with these observations, a transgenic-overexpression strategy leading to increased serum-levels of IFN-λ3 resulted in increased systemic bacterial loads, tissue injury and mortality in these mice.

Discussion/Conclusion: In conclusion, the present data suggest that activation of IFN-λ/IFN-λR signaling contributes significantly to bacterial sepsis and this could have implications for human disease.
Intestinal epithelial Stat3 activation controls *C. rodentium* infection

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**Introduction:** We have previously shown that conditional knockout mice lacking the ability to activate the transcription factor Stat3 specifically in intestinal epithelial cells (IECs) show decreased secretion of antimicrobial peptides and deregulated IEC homeostasis. We now investigated the role of Stat3 activation in IECs during gastrointestinal infections.

**Methods:** For investigation of IEC Stat3 activation during intestinal infections, IEC-specific Stat3 conditional knockout mice (Stat3^IEC-KO^) were analysed using infection with *Citrobacter rodentium*, a murine model pathogen for attaching and effacing *Escherichia coli* such as EHEC and EPEC in men.

**Results:** We could show that infection of wildtype mice with *C. rodentium* resulted in rapid activation of Stat3 in colonic epithelial cells. *Citrobacter*-infected wildtype mice showed strong induction of antimicrobial peptides concurrent with epithelial activation of Stat3. Conditional knockout mice with an IEC-specific deletion of Stat3 revealed an increased susceptibility to *Citrobacter* infection as indicated by elevated bacterial load in the gut and more severe colitis. Furthermore, histological examination of colonic tissue samples from *Citrobacter*-infected Stat3^IEC-KO^ mice demonstrated enlarged infiltration of immune cells, enhanced mucosal hyperplasia and increased epithelial apoptosis. Strikingly, Stat3^IEC-KO^ mice showed unexpected invasion of *Citrobacter* into other organs such as lung, liver and kidney, indicating a defective mucosal barrier.

**Discussion/Conclusion:** These data implicate a protective role for intestinal epithelial Stat3 activation during *Citrobacter* infection by controlling bacterial growth in two different ways: production of antimicrobials and suppression of apoptosis for maintaining the epithelial barrier integrity.
Interrelation between the functional state of erythrocytes and morphological parameters in children with inflammatory bowel disease

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Introduction: The aim of this study was to investigate interrelation between the functional state of erythrocytes and morphological parameters in children with inflammatory bowel disease, which define the inflammation process activity.

Methods: Erythrocyte membrane permeability was investigated by urea hemolysis (Kolmakov, 1984) in 49 children with ulcerative colitis and 31 children with Crohn’s disease. The erythrocyte membrane permeability was estimated according to urea concentration in effecting solution. The control group consisted of 30 healthy children. At the same time in 24 patients with ulcerative colitis and 12 children with Crohn’s disease, the large bowel mucosa morphometric study was conducted. Received data was analyzed using the Mann-Whitney U test by STATISTICA 8.0 Stat. Soft Inc. (1984–2007).

Results: Children with ulcerative colitis were divided into two groups by value of hemolysis degree. Statistically significant difference between the first group (children with low hemolysis degree) and the control group was found by the level of erythrocyte membrane permeability changing. In the first group moderate mononuclear infiltrate lymphocytes and plasmocytes were detected in equal proportions. In proper mucous plate fibroblastic series cells was detected in raised quantity, and that fact indicated the second degree of histological activity. In the second group (children with high hemolysis degree) the maximal cell density and crypt-abscesses were founded with erythrostasis and expressed leukodiapedesis, histological activity was estimated as the 3–4 degree. Children with Crohn’s disease were divided into three groups by value of hemolysis degree. Statistically significant difference between the first group (children with low hemolysis degree) and the third one (children with high hemolysis degree) was found as well as between the third and the second group (children with medium hemolysis degree). Morphometric characteristics of the large bowel mucosa were the same by histological activity parameters (cell density evidence and infiltrate composition) for the first and the third children groups. These children were characterized by intraepithelial lymphocytes raising content, ill-defined lymphoplasmacytic infiltration, lymphocyte accumulation by type of lymphoid follicles and their frequent presence, as well as the foci of fibrosis were identified. Thereby, erythrocyte membrane state changes might serve as an inflammation activity marker with similar morphological parameters. In patients whit expressed mucosa destruction, muscle plate dissociation and other inflammation processes activity markers beyond lymphoid tissue, the high hemolysis degree was always detected. Thereby, hemolysis degree reflects changes at system organ level.
Discussion/Conclusion: Erythrocytes functional state disorders were detected in children with inflammatory bowel disease. Degree of erythrocyte membrane permeability serves as an index of inflammatory process activity in bowels mucosa without reference to nosology.
The role of capsule endoscopy in evaluating and managing small bowel disorders

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Introduction: The small bowel has always been a challenging area for the gastroenterologist to investigate. Currently, no gold standard exists; a combination of methods is used to diagnose small bowel pathology. Capsule endoscopy (CE), is an innovative, non-invasive method of visualising the entire small bowel, however, it is not routinely performed due to expense. 58 CE patients from the Royal Bolton Hospital were audited against guidelines based on the British Society of Gastroenterologists (BSG) 2007, reviewing whether this investigation has a positive influence in clinical outcome.

Methods: Case notes of those requiring CE from January 2007–June 2011 were studied retrospectively. Inclusion criteria included a negative oesophagaelgastro-duodenoscopy, colonoscopy, or barium follow-through. The indication, presenting complaint, and blood transfusion requirement were all noted, as were results of all prior investigations.

Results: CE confirmed a diagnosis in 15 (26%). The cause for obscure gastrointestinal bleeding was discovered in 11 (29% of patients indicated), Crohn’s disease (CD) was confirmed in 3 (21%). It found a cause for chronic abdominal pain in one patient (2%), which was CD. Abnormal results were discovered in 19 (33%); which were found to angiodysplasia (7), CD (6), polyps (2), nodular lymphoid hyperplasia (1), distorted villi (1), coeliac (1), ulceration (1).

Discussion/Conclusion: The diagnostic yield is comparable to other studies carried out with similar sample sizes. CE found the cause of OGID in 29% and confirmed CD in 21% indicated, when other modalities could not, influencing clinical outcome. There may be a more prominent role for CE in future clinical practice.
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