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SELECTION OF THE MONTH

Big Title: Gut-Liver interaction in PSC

Small titles:

Sarcopenia and fibrosis in NAFLD

Trends in global HBV epidemiology

Dynamics of HCV replication organelles generation

ACUTE LIVER INJURY

Kruppel-like factor 2 and autophagy cooperate to protect the endothelium

The transcription factor kruppel-like factor 2 (encoded by *KLF2*), which is induced by simvastatin (among other inducers), promotes endothelial protection. Guixé-Muntet *et al.* hypothesized a role of autophagy in kruppel-like factor 2-mediated endothelial protection. They now show that stimulation of autophagy results in kruppel-like factor

2 overexpression which accentuates autophagy by a positive feedforward mechanism. Acute liver injury caused by ischemia/reperfusion (I/R) inhibits the autophagy/kruppel-like factor 2 cooperation and results in endothelial cell death. **Interestingly, simvastatin-pretreatment protects mice against I/R-associated endothelial injury because of sustained autophagy and kruppel-like factor 2 expression and subsequent endothelial cell survival.** Because I/R is an important issue following liver transplantation, the findings by Guixé-Muntet and colleagues suggest that simvastatin could be useful in preventing this complication.

CHOLESTATIC LIVER DISEASE

Parsing the phenotype of *ABCB11* deficient mice

Impairment of the canalicular bile acid (BA) transport via the bile salt export pump (encoded by *ABCB11*, also known as *BSEP, PFIC2*) causes cholestasis. Absence of bile salt export pump in humans causes progressive familial intrahepatic cholestasis type 2, a severe cholestatic liver disease in children. In contrast to humans, *Abcb11* deficiency in mice is associated with a milder phenotype lacking the development of progressive cholestasis. This finding may at least in part be explained by differences in BA composition, metabolism and transporters between mice and men. In this issue of the Journal, Fuchs *et al.* addressed the question by investigating wild type and *Abcb11*$^{-/-}$ mice that were subjected to common bile duct ligation or 3.5-diethoxycarbonyl-1.4dihydrocollidine (DDC) feeding as models for cholestasis with biliary obstruction and bile duct injury. They show that **mice with inborn *Abcb11* deficiency exhibit an adaptive increase of polyhydroxylated BAs that may precondition and thereby protect against acquired cholestatic liver and bile duct injury.**

CHOLANGIOCARCINOMA

Cancer stem cells (CSCs) shape tumor-associated macrophages (TAM)

CSCs play a crucial role in clinical severity of cholangiocarcinoma. Macrophages are not only involved in tissue homeostasis and immune defense against pathogens, but also can be regulators of cancer, being called TAM in this case. Raggi et al. hypothesized that CSCs may induce a tumor-promoting TAM phenotype. They reveal that **CSCs shape tumor-initiating niche by educating associated macrophages via signals such as interleukin (IL)-13 (known to promote M2 phenotype), IL-34**

(known to stimulate monocytes via the M-CSF receptor) and osteoactivin (encoded by *GPNMB*, for transmembrane glycoprotein NMB, whose functions are elusive).

HEPATOCELLULAR CARCINOMA (HCC)

Dissecting anti-tumor immunity, No touch multi-bipolar radiofrequency *vs.* mono-polar techniques for small HCC

Dissection of antitumor immunity during tumor initiation and progression has been a challenge in the absence of clinically relevant animal models. Li *et al.* report very important new findings in the current issue of the Journal. First, they show that a combination of intraperitoneal injection of carbon tetrachloride and intra-splenic inoculation of oncogenic hepatocytes is able to induce progressive HCCs in fibrotic livers of immunocompetent mice. This model recapitulates main features of human HCC. Second, using this new animal model, they find that both immunosuppressive regulatory T cell (**Treg**) accumulation and upregulation of programmed cell death protein 1 (i.e., **protein PD-1**, whose engagement at $CD8^+$ T cell surface inhibits the cytotoxic, anti-tumoral action of these cells), are two independent mechanisms inducing profound immune tolerance in HCC. Third, **using this model, they reveal that therapy using a combination of sunitinib (a novel small molecule that blocks multiple receptor tyrosine kinase) with antibodies against protein PD-1 achieves significant tumor control, supporting translation of this approach for the treatment of patients with HCC.**

Although very encouraging results have been reported after treatment of small HCC with multi-bipolar radiofrequency ablation (RFA), the mono-polar technique remains the most frequently used technique worldwide. Hocquelet *et al.* compared the rate of RFA failure between mono-polar RFA and “No Touch” Multipolar RFA for the curative treatment of small HCC (≤5cm) in a large multi-center case-matched study. **They show that in the context of HCC ≤5 cm, “No Touch” Multipolar RF provides better primary success and sustained local tumor response without increasing severe complications rates.**

NON-ALCOHOLIC STEATOHEPATITIS (NAFLD)

Effect of aerobic vs resistance physical exercise and role of sarcopenia and PARylation

Recent studies suggest that sarcopenia is present in many patients with NAFLD. In this issue, Koo *et al.* studied the appendicular skeletal muscle mass (ASM) in a cohort of patients with NAFLD. The prevalence of sarcopenia in subjects without NAFLD, simple steatosis and NASH were 9%, 18%, and 35%, respectively. The **degree of sarcopenia inversely correlated with the severity of fibrosis** and was associated with significant fibrosis independent of body mass index and insulin resistance. Moreover, among NAFLD subjects, subjects with sarcopenia were more likely to have NASH than those without sarcopenia. This clinical study highlight that sarcopenia is associated with severe stages of NAFLD. Further clinical and translational studies should reveal the mechanisms and potential non-specific ad targeted therapies. Another interesting article in this issue of the *Journal* comparatively analyzes the existing evidence of aerobic (short, more intense) *vs.* resistance (more maintained) exercise in NAFLD. Exercise is a first-line therapy for patients with NAFLD. Hashida *et al.* performed an extensive search of and found 95 published studies on the role of exercise in these patients. Aerobic vs resistance exercises were compared. **Both aerobic and resistance exercise improved hepatic steatosis.** No significant difference was seen in the duration, frequency, or period of exercise between the two exercise groups. However, resistance exercise improves NAFLD with less energy consumption. Thus, **resistance exercise may be more feasible than aerobic exercise for NAFLD patients with poor cardiorespiratory fitness** or for those who cannot tolerate or participate in aerobic exercise. This study suggests a possible link between resistance exercise and lipid metabolism in the liver.

Finally, an interesting experimental study in this issue reveals a novel mechanism of NAFLD that could represent a new therapeutic strategy. Oxidative stress is an important feature leading to NAFLD. Because **poly-ADP ribosylation (PARylation) of proteins by polymerases (PARPs) consumes NAD+,** Gariani *et al.* hypothesized that over-activation of PARPs drives NAD+ depletion in NAFLD. To test this hypothesis, examined the preventive and therapeutic benefits of the PARP inhibitor, olaparib, in different models of NAFLD. **PARP inhibition reversed NAFLD by repletion of NAD+, and reduced reactive oxygen species, ER-stress and fibrosis**. This effect was dependent on the activation of sirtuin-1, an epigenetic regulator. This interesting study proposes that targeting PARylation could be beneficial in patients with NAFLD.

HEPATITIS C VIRUS (HCV) INFECTION

Mortality risk in patients cured from their HCV infection, real-world results of sofosbuvir plus daclatasvir in HCV type 1, dynamics of HCV replication organelles generation, no evidence for intra-hepatic HCV compartmentalization in late stage liver disease

The number of people living with cured HCV infection is expected to grow rapidly in the era of highly effective antiviral regimens. Although treatment-induced cure of the infection (sustained virologic response, SVR) is associated with improved patient survival, the prognosis after SVR relative to the general population is unclear. The study by Innes *et al.* identified 1,824 patients, followed on average for 5.2 years after SVR, using a national Scottish database which includes cause-specific mortality data, and compared the frequency of mortality in SVR patients to the general population. **Overall, all-cause mortality was 1.9 times more frequent for SVR patients than the general population, and mainly driven by death from liver cancer and drug-related causes which accounted for 66% of the total excess death observed**. As all modifiable characteristics associated with increased mortality were markers of either heavy alcohol or injecting drug use, and individuals without these behavioral markers (33% of the cohort) experienced equivalent survival to the general population, this study highlights the importance of a multidisciplinary approach targeting health risk behaviors in the SVR population. Identifying those SVR patients who might benefit from regular HCC screening remains a further future challenge.

In clinical trials the combination of the pan-genotypic NS5A inhibitor daclatasvir with the pan-genotypic nucleosidic polymerase inhibitor sofosbuvir has demonstrated high antiviral efficacy against chronic HCV infection. Pol *et al.* report now the first real-world results from their French multicenter observational cohort which included 768 HCV type 1-infected patients treated with sofosbuvir plus daclatasvir with or without ribavirin for a duration of 12 weeks or 24 weeks. **The SVR ranged from 92% in the 12-week ribavirin-free regimen to 99% in the 24-week ribavirin-containing regimen.** A greater than 97% SVR rate was observed in patients without cirrhosis irrespective of the treatment duration whereas among patients with cirrhosis extending treatment duration to 24 weeks increased SVR rates from 88% (12 weeks) to 95%. This large-scale study confirms the high efficacy of the regimen in the real-life setting but provides also relevant information concerning the need for adding ribavirin as well as individualizing treatment duration according to the stage of fibrosis.

Like all positive-sense RNA viruses, HCV induces cytoplasmic membrane alterations in infected cells, which have been termed ‘replication organelles’. The viral nonstructural NS5A protein is essential for their formation, and they are believed to be sites of HCV RNA synthesis. There remain, however, many questions regarding the temporal regulation, turnover and composition of HCV replication organelle function in chronically infected cells. In this elegant study, Wang and Tai used a pulse-chase fluorescent labeling approach that allows to discriminate ‘old’ from ‘new’ NS5A-positive membranous structures (NS5A foci) herby **demonstrating that HCV replication membrane structures are continuously generated at spatially distinct sites supporting a model of continuous *de novo* replication organelle formation instead of resupply of previously-formed ones**. Whereas lipid kinase phosphatidylinositol 4-kinase IIIα (PI4KA), oxysterol-binding protein (OSBP), and NS5A are required to initiate ‘new’ NS5A foci, cholesterol is preferentially trafficked to ‘old’ NS5A foci and is required for association of ‘old’ foci with HCV core protein and lipid droplets. The study provides important new insights in the generation and dynamics of viral replication organelles which may be associated with changes in their function and morphology, hereby potentially modifying viral infection cycle.

The primary site of HCV replication is the liver, and recent studies suggest that 1 to 50% of the hepatocytes are infected. These infected cells occur as clusters surrounded by uninfected hepatocytes, supporting a model of intra-hepatic HCV compartmentalization, where viral variants may be localized to discrete regions of the liver. To investigate the spatial influence of the liver architecture on viral replication, and whether local interferon responses may limit HCV replication and evolution, Hedegaard *et al.* measured HCV RNA and interferon-stimulated gene (ISG) expression from each of the 8 Couinaud liver segments from 21 patients undergoing liver transplantation. **The levels of HCV RNA in all eight biopsies sampled from a single liver as well as hepatic and plasma viral quasi-species were surprisingly similar, suggesting that the liver is uniformly infected during end-stage liver disease**. By using high resolution HCV sequencing and monitoring of innate immune responses at multiple sites across the liver, a uniform pattern of diversity was identified **which argues against viral compartmentalization**. The study also provides novel insights into the relationship between hepatic ISG expression and the viral quasi-species in the liver and periphery.

HEPATITIS B VIRUS (HBV) INFECTION

Long-term safety and efficacy of tenofovir in lamivudine-resistant HBV infection, trends in global HBV epidemiology

Although tenofovir monotherapy has proven activity in lamivudine-resistant HBV infection, long-term controlled studies are missing showing its efficacy and safety in comparison to a combination approach, an approach which was believed to be more effective in the resistance setting. The randomized-controlled study by Fung *et al.* is the first presenting long-term efficacy and safety results, after 5 years of tenofovir monotherapy or tenofovir plus emtricitabine combination in 280 patients with lamivudine-resistant HBV infection. **At week 240 no difference was observed between the 2 groups regarding HBV DNA clearance, ALT levels, HBsAg and HBeAg loss, and no patient developed resistance-associated mutations in the tenofovir monotherapy group**. The risk of renal dysfunction was low with a 7% rate of confirmed creatinine clearance of less than 50 mL/min. This is also the first study to prospectively evaluate changes in bone mineral density (BMD) from baseline using serial DEXA scans. The proportion of patients with BMD T-scores consistent with osteopenia and osteoporosis increased from baseline to week 240 by 6% points for the spine and 8% points for the hip. The study proofs the high efficacy of the monotherapy approach with tenofovir in lamivudine-resistant HBV infection, and the overall low – but not negligible - risk of mild kidney dysfunction and osteopenia/osteoporosis development in the long-term.

A better knowledge of country-specific trends in HBV prevalence may provide important information concerning our previous actions undertaken in order to combat this worldwide leading viral infection but will also tell us whether further prevention strategies are necessary. Based on published data, Ott *et al.* applied a linear model on the logit scale to assess time trends in estimated HBsAg prevalence in 2000 and their relative changes over time by country and region. **In most of the 50 countries with sufficient data, a decrease in HBsAg prevalence was seen over time. The time changes in HBsAg prevalence in the WHO European region revealed four different patterns**: stable or even increase (Russia, Poland, and Romania), no to medium reduction (France, Germany, Israel, the Netherlands, Italy, and Spain), medium relative decrease (Albania and Turkey), and strong relative decrease (Greece, Slovenia, and United Kingdom). Decreases in HBsAg prevalence occurred mostly before direct effects of childhood vaccination may manifest indicating preventive

effects of various other health measures. The findings of stable or even increasing HBsAg prevalence in some countries of Africa and Eastern Europe underline the importance of ongoing and tailored HBV prevention activities and can further assist implementing WHO`s prevention and control recommendations.

PRIMARY SCLEROSING CHOLANGITIS

Memory T-cells of common clonal origin involved in PSC-IBD pathogenesis

The immunologic mechanisms involved in the pathogenesis of PSC-IBD are unclear and the current hypotheses suggest that T-cells recruited into the liver from the gut may drive hepatic inflammation. Henriksen *et al.* studied liver and colonic biopsies to determine whether the T-cells obtained from these organs share common receptors and antigenic specificities. **The authors used high throughput sequencing and made the novel observation that in the PSC-IBD patients, memory T-cells of common clonal origin was detected in the paired biopsies suggesting that memory T-cells driven by shared antigens may be important in the pathogenesis.** These data allow potentially novel approaches to therapy targeting the memory T-cells.