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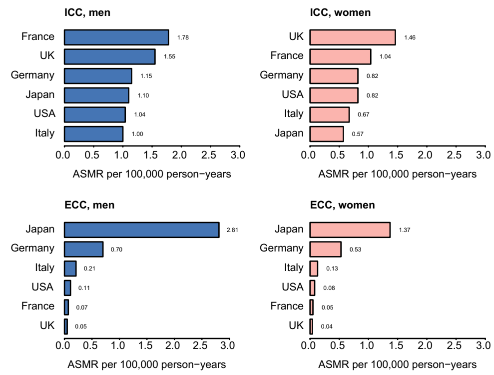
From the Editor's desk. . .

Richard Moreau*, Ramon Bataller, Thomas Berg, Sophie Lotersztajn, Jessica Zucman-Rossi, Rajiv Jalan

SELECTION OF THE MONTH

Increasing mortality from intrahepatic cholangiocarcinoma

Intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC) have rarely been studied separately. Mortality trends from these 2 neoplasms have been inconsistent over the last decades. **Bertuccio *et al.*** aimed to analyse trends in mortality from ICC and ECC in selected countries worldwide. For this, they extracted death certification data for ICC and ECC and population estimates from the World Health Organization and Pan American Health Organization databases for 32 selected countries from Europe, the Americas, and Australasia over the 1995-2016 period. **Here, they reveal a global increase in mortality from ICC – possibly due, in part, to better disease classification, as well as to trends in risk factors.** Mortality from ECC levelled off or decreased, most likely following the increased use of laparoscopic cholecystectomy.



Bertuccio *et al.*, 2019.
Increasing mortality from intrahepatic cholangiocarcinoma

LIVER HOMEOSTASIS

Role of mechanotransduction in hepatocyte homeostasis

Cell function can be regulated by the mechanical properties of cells and of the microenvironment. Cells measure these features by developing forces via their actomyosin cytoskeleton and respond by transducing forces into biochemical signals that instruct cell behaviour. Among these, the transcriptional coactivators yes-associated proteins (YAP)/transcription activator with PDZ-binding motif (TAZ) recently emerged as key factors mediating multiple responses to actomyosin contractility. However, whether mechanical cues regulate adult liver tissue homeostasis, and whether this occurs through YAP/TAZ, is poorly understood. **Pocaterra *et al.* now reveal a previously unrecognised role for the F-actin barbed end capping protein CAPZ (F-actin-capping protein subunit beta) in tuning the mechanical properties of cells and tissues, which is required in hepatocytes for the maintenance of the differentiated hepatocyte state and to regulate organ size.** Of note, this study indicates for the first time a physiological role of mechanotransduction in maintaining tissue homeostasis in mammals.

CONGENITAL HEPATIC FIBROSIS

Loss of fibrocystin induces interleukin-8-dependent proliferation of biliary epithelium

Congenital hepatic fibrosis (CHF) is a genetic liver disease characterised by abnormal proliferation of cholangiocytes and progressive hepatic fibrosis. CHF is caused by mutations in the polycystic kidney and hepatic disease 1 (*PKHD1*) gene. Mutated *PKHD1* results in dysfunction of its protein product, fibrocystin, which is associated with ductal plate malformation during foetal development and further pathological changes in patients with CHF. However, the underlying molecular mechanism of CHF remains unclear, which is quite different from liver cirrhosis. Tsunoda *et al.* aimed to investigate the molecular mechanism of CHF-pathophysiology using a genetically engineered human induced pluripotent stem cell model to aid the discovery of novel therapeutic agents for CHF. They uncover that **loss of fibrocystin function promotes interleukin (IL)-8-dependent proliferation of connective tissue growth factor (CTGF)-producing cholangiocytes, suggesting that IL-8 and CTGF are essential for the pathogenesis of CHF.** IL-8 and CTGF are candidate molecular targets for the treatment of CHF.

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

p38 MAPK mediates NASH by promoting M1 macrophage polarisation

Previous studies indicate that macrophage polarisation towards an inflammatory phenotype (M1) plays a role in non-alcoholic steatohepatitis (NASH). The mechanisms underlying this cellular event are unclear. In this issue, **Zhang *et al.*** studied the role of p38 mitogen-activated protein kinases (MAPKs), which typically promote tissue inflammation. In a highly translational study including human and experimental data, the authors demonstrate that p38α was significantly upregulated in liver tissues of patients with NAFLD. At the experimental level, p38αΔHep mice developed significant nutritional steatohepatitis in 3 models of NASH compared to their littermates. The effect of macrophage p38α in promoting steatohepatitis was mediated by the induction of pro-inflammatory factors secreted by M1 macrophages and associated signalling pathways. In fact, p38αΔMφ mice exhibited M2 anti-inflammatory macrophage polarisation. These *in vivo* data were corroborated *in vitro* using a co-culture system of hepatocytes and macrophages. This interesting study provides evidence that macrophage p38α promotes the progression of NASH by inducing pro-inflammatory cytokine secretion and M1 polarisation. p38 inhibition represents an appealing strategy to protect against diet-induced NASH.

HEPATITIS A VIRUS (HAV) INFECTION

Host-specific innate immunity determines cross-species transmission

HAV likely entered human populations only 10-12 thousand years ago after jumping from a rodent host. The prerequisites required for such cross-species transmission are largely unknown. However, as viruses have evolved

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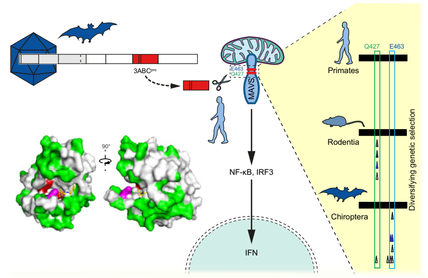


Feng *et al.*, 2019.
Host-specific innate immunity determines cross-species transmission

mechanisms to evade innate host-specific antiviral defences, **Feng *et al.*** speculated that the capacity to overcome innate antiviral responses in a foreign species is likely key. A critical innate immune adaptor protein is mitochondrial antiviral signaling protein (MAVS), which is cleaved by the precursor of the mature HAV $3C^{pro}$ protease, 3ABC, hereby abrogating innate immune responses in the liver. Several lines of evidence suggest that HAV must overcome MAVS-mediated innate immunity to achieve cross-species transmission, and the authors therefore asked whether the 3ABC proteases of contemporary bat and rodent viruses are capable of cleaving human MAVS. **The authors show that distantly related hepatoviruses that infect bats and rodents today express proteases that disrupt innate antiviral responses in human cells, leading to the intriguing question of whether contemporary bat viruses pose a risk to human populations**.

HEPATITIS B VIRUS (HBV) INFECTION

Tenofovir monotherapy stands against resistance

Tenofovir (TDF) monotherapy has proven high activity against both wild-type and lamivudine-resistant HBV infection but it has remained uncertain whether similar efficacy can be achieved in the long-term when given as monotherapy for adefovir (ADV)- or entecavir (ETV)-resistant HBV infection. In this issue of the *Journal*, the 5-year virologic efficacy and safety of TDF monotherapy was investigated in heavily-pretreated patients with multidrug-resistant HBV infection who were previously involved in 2 prospective randomized controlled trials. **Lim *et al.*** showed that **although the virologic response rate in the patients with ADV resistance was slower than that in those with ETV resistance, most patients showed effective viral suppression with TDF monotherapy at week 240, and additional resistance-associated mutations did not emerge in any patients**. However, serologic response rates were quite low and starting after week 144, a progressive and significant decrease in estimated glomerular filtration rate (eGFR) and femoral (but not spine) bone marrow density was observed. This long-term study provides clear-cut evidence of the high barrier against viral resistance associated with TDF monotherapy. However, as serologic response rates were low in the multidrug-resistant HBV infection, long-term TDF administration was required, raising concerns about the kidney and bone safety of TDF. Therefore, studies have already started evaluating the safety and efficacy of tenofovir alafenamide, a drug which may provide improved renal and bone safety than TDF, in patients with multiple drug-resistant HBV.

HEPATITIS C VIRUS (HCV) INFECTION

Scaling up DAA treatment in PWID – adherence matters, the sensitivity that is needed for POC HCV RNA testing, long-term protection against chronic infection by neutralising antibodies, E2-containing exosomes – a new mechanism to escape neutralising antibody response

Scaling up treatment for HCV-infected people who inject drugs (PWID) is of upmost importance to effectively impact the HCV epidemic worldwide. However, concerns exist that PWID, whether on opiate agonist therapy (OAT) or not, might achieve lower cure rates either because of lower adherence or higher HCV-reinfection rates. From 2 large prospective multicentre cohorts of patients receiving direct-acting antivirals (DAAs) in clinical practice in Spain, the HEPAVIR-DAA and GEHEP-MONO cohort, **Macías *et al.*** evaluated sustained virologic response (SVR) and reinfection rates among a total of 1,752 patients, who were either non-drug users or PWID with and without OAT. **Compared to patients who never injected drugs, the SVR rates were significantly lower in PWID with or without OAT (95% *vs.* 89% or 92%, respectively), and the lowest SVR12 rates were observed in ongoing drug users regardless of OAT use (79%).** Loss to follow-up was the most common reason for not achieving SVR in the intention-to-treat analysis and among ongoing drug users 3.5% became reinfected. Although the study shows that guaranteeing adherence to treatment and follow-up evaluations remain an area for improvement when treating PWID, it clearly confirms the high efficacy of DAAs in this patient population. Even in the most difficult-to-treat group of patients with ongoing drug use, a nearly 80% chance for cure – although far from optimal – will significantly impact the infection chain among active PWID.

Implementation of point-of-care (POC) HCV RNA assays is crucial when trying to realise test and treat strategies to improve access to HCV treatment, especially in middle- and low-income countries. Affordable POC HCV RNA tests are already on the market but do not currently achieve the same prequalification sensitivity criteria with respect to the limit of detection as laboratory based real-time PCR HCV RNA assays, which have a very high sensitivity with a limit of detection threshold of approximately 10 IU/ml. As this high sensitivity is probably not needed when trying to screen and large cohorts of untreated patients, **Freiman *et al.*** created a dataset of 66,640 participants with HCV infection from 12 countries, representative of 6 global regions, in order to evaluate the distribution of viral loads to inform a requisite limit of detection for a POC test that could capture >97% of those with viraemic infection. **The authors found that low-level viraemia is infrequent in untreated patients, regardless of subgroup characteristics and the studied region, and that a limit of detection of 1,300 IU/ml, 100 times higher than the current gold-standard PCR diagnostic test, remained 97% sensitive to diagnose viraemic HCV infection**. This important large-scale study provides a good rationale for implementing less strict prequalification sensitivity criteria with respect to the limit of HCV RNA detection when developing affordable POC tests to expand HCV testing and linkage to care in low- and middle-income countries.

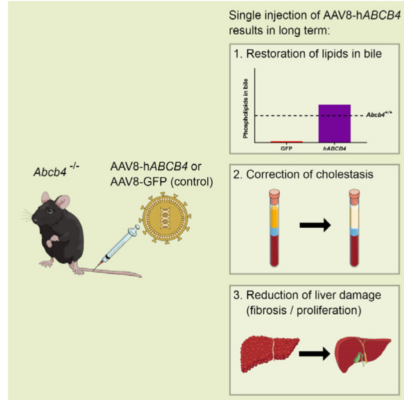
Patients with ongoing risk behaviours who spontaneously cleared an episode of acute HCV infection remain at risk of HCV reinfection. With every episode of spontaneous HCV clearance, however, the likelihood of becoming reinfected decreases, indicating that, at least to some extent, a protective immunity also exists in HCV infection. Indeed, broadly-reactive, neutralising antibodies have been isolated from B cells of HCV-infected individuals, but it remains unclear whether B cells producing such antibodies contribute to the clearance and long-term immune protection against HCV. **Merat *et al.*** now analysed the B-cell repertoire among injecting drug users from the Amsterdam Cohort Study who became chronically infected either after primary infection or after reinfection, and those who spontaneously cleared HCV after 1 or multiple infections. In spontaneous clearers, they were able to demonstrate the presence of a high frequency of cross-genotype antibodies mainly directed against the antigenic region 3 of the HCV envelope proteins (E1/E2) that showed cross-neutralising capacity. **This elegant study describes for the first time, an early and strong B-cell response that induces broadly neutralising antibodies, especially targeting antigenic regions 3 and 4 of the HCV envelope protein, contributing to HCV clearance and long-term immune protection against HCV, a finding which may have major implications for vaccine development.**

Due to its intimate association with host-derived components of very low-density lipoproteins and low-density lipoproteins, infectious HCV particles represent hybrid virions designated lipo-viro-particles (LVPs). Besides the non-exchangeable apolipotein (apo) B and several exchangeable apolipoproteins (apoE, AI, CI and CIII), multiple other cellular proteins were found to be specifically associated with/incorporated into envelope protein 2 (E2)-containing LVP particles. By demonstrating that many of these proteins share common markers of exosomes, in particular syntenin, previous studies suggest that E2 might be a component of exosomes. Given the pivotal role of syntenin in exosome biogenesis and its presence in E2-containing particles, **Deng *et al.*** studied the role of syntenin in the HCV life cycle. **The authors show that syntenin expression promotes the release of E2-containing exosomes that contribute to escape neutralisation by both E2 specific antibody and chronic-phase patient serum which renders hepatitis C virions less susceptible to neutralising E2-specific antibodies.** The authors elegantly describe a new strategy HCV utilises to escape neutralising antibody responses, which poses another challenge for rational HCV vaccine development.

CHOLESTASIS

Gene therapy for familial cholestasis

Progressive familial intrahepatic cholestasis (PFIC3) is a devastating disease with limited therapeutic options. Technological improvements have led to liver-targeted gene therapy becoming a clinical reality for a multitude of clinical conditions. **Aronson *et al.* describe the results of an innovative approach, using adeno-associated virus serotype 8 (AAV8)-mediated gene therapy directed to the liver to correct the ABCB4 transporter defect, which is a feature of PFIC type 3.** They performed these experiments in an *Abcb4* deficient mouse model. Their data show that this approach led to stable transgene expression, which restored biliary phospholipid excretion and resulted in normalisation of plasma cholestatic markers. This prevented progressive liver fibrosis. The data provide proof of concept, which can be further developed for translation into man.



Aronson *et al.*, 2019.
Gene therapy for familial cholestasis

LIVER TRANSPLANTATION

Predictors of liver failure following surgical correction of biliary atresia in adolescence

In patients with biliary atresia treated with the Kasai procedure in childhood, about 14–44% of patients will survive into adulthood with their native liver, a proportion of whom will need a liver transplant. Factors determining which patients will need a liver transplant when >16 years old are not known. **Jain *et al.* performed a large retrospective study in patients operated for biliary atresia in a single centre between 1980 and 1996. They observed that it was not possible to develop a prognostic model but identified a bilirubin of 21 µmol/l or more, presence of portal hypertension and cholangitis and low creatinine in adolescence as potential markers of prognostic value.** These data are valuable in identifying patients at high-risk of needing a liver transplant, enabling better management.

LIVER CANCER

Inhibition of histone modifiers Jumonji lysine demethylases for treating HCC, outcome of HCC with immune cell stroma

The nucleosome is the fundamental unit of chromatin. It is composed of an octamer of the 4 core histones (H3, H4, H2A, H2B) around which 147 base pairs of DNA are wrapped. Several distinct classes of enzyme can modify histones at multiple sites; these enzymes include acetyltransferases, lysine methyltrasferases, and lysine demethylases (now known as K-demethylases [KDMs]; the paradigm being Jumonji lysine demethylases [KDM2A, 2B, 3A, 3B, 4A to 4D]). The modifications these enzymes mediate play important roles in epigenetic processes involving DNA manipulation, such as transcription, repair, and replication. Because epigenetic alterations are likely to be involved in hepatocarcinogenesis, these alterations may be targets for therapeutic interventions. **Bayo *et al.*** aimed to identify and target epigenetic modifiers that show molecular alterations in HCC. Using elegant approaches, they show that mutations and changes in expression of epigenetic modifiers are common events in HCC, leading to an aggressive gene expression programme and poor clinical prognosis. **The transcriptional programme can be reversed by pharmacological inhibition of Jumonji enzymes, providing a novel potential therapeutic approach for HCC.**

The immunogenomic characteristics of histologically defined HCC with immune cell stroma (HCC-IS) have not been clarified. To address this question, **Kang *et al.*** investigated the clinical and molecular features of HCC-IS and the prognostic potential of Epstein-Barr virus (EBV) infection. They evaluated 219 patients with conventional HCC and 47 with HCC-IS using *in situ* hybridisation for EBV, immunohistochemistry, multiplex immunofluorescence staining, and whole exome and transcriptome sequencing. Genomic and prognostic parameters were compared between HCC-IS and conventional HCC. They now show that **HCC-IS is a distinct HCC type associated with a good prognosis and frequent presence of EBV-positive tumour-infiltrating lymphocytes. Paradoxically, a higher density of EBV-positive tumour-infiltrating lymphocytes acted as a negative prognostic factor. HCC-IS could be candidates for immunotherapy.**

Accuracy of ^{18}FDG-PET in biliary tract cancers

The role of ^{18}F-fluorodeoxyglucose positron emission tomography (18FDG-PET) in the diagnosis and staging of patients with biliary tract cancer remains controversial. **Lamarca *et al.*** performed a systematicreview and meta-analysis of 47 eligible studies with 2,125 patients to explore the diagnostic test accuracy of ^{18}FDG-PET in the diagnosis of primary tumour, lymph node, distant metastases and relapsed disease. **They show evidence for including ^{18}FDG-PET in the current standard of care staging imaging/diagnostic tests performed for the assessment of lymph nodes, distant metastasis and relapse to guide adequate treatment selection, especially if the identification of occult sites of disease would change management (*i.e.*, surgery/local therapies) or if diagnosis of relapse remains unclear following standard of care imaging.** The use of ^{18}FDGPET for diagnosis of primary tumours in the absence of other disease sites or pathological confirmation remains controversial, due to low specificity.

Richard Moreau* at Centre de Recherche sur l'Inflammation (CRI), INSERM, Université Paris Diderot, Paris, France; DHU UNITY, Service d'Hépatologie, Hôpital Beaujon, Assistance Publique-Hôpitaux de Paris, Clichy, France; Laboratoire d'Excellence (Labex) Inflamex, COMUE Sorbonne Paris Cité, Paris, France.
* Corresponding author. *E-mail address:* richard.moreau@inserm.fr
Ramon Bataller at Division of Gastroenterology, Hepatology and Nutrition University of Pittsburgh Medical Center, Pittsburgh, PA, USA.
Thomas Berg at Section Hepatology, Clinic for Gastroenterology and Rheumatology, University Hospital Leipzig, Leipzig, Germany.
Sophie Lotersztajn at Centre de Recherche sur l'Inflammation (CRI), INSERM, Université Paris Diderot, Paris, France.
Jessica Zucman-Rossi at Inserm UMR-674; Génomique Fonctionnelle des Tumeurs Solides; IUH; Paris, France; Université Paris Descartes; Labex Immuno-oncology; Faculté de Médecine; Sorbonne Paris Cité; Paris, France.
Rajiv Jalan at Liver Failure Group, Institute for Liver and Digestive Health, University College London, Royal Free Hospital, UK.