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View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.26961
Potential conflict of interest: Dr. Chen consults for, advises, and is on the speakers' bureau for Bristol-Myers Squibb, Roche, and Gilead.

Hepatocyte Levels of CD73 Contribute to Mallory-Denk Body Formation

To the Editor:

Snider et al.[1] reported low levels of CD73 in hepatitis C and nonalcoholic steatohepatitis (NASH) livers, where Mallory-Denk bodies (MDBs) formed. In a mouse model (C57BL), low levels of CD73 developed when fed 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) and MDBs formed. Thus, both human and mouse livers with reduced levels of CD73 correlated with increased MDB formation. Paradoxically, Snider et al.[1] observed that $CD73^{-/-}$ mice fed DDC failed to form MDBs. They offer no mechanism to explain this paradox. "If low levels of CD73 increase MDB formation, isn't it logical to expect that, in the absence of CD73 in $CD73^{-/-}$ mice, they would form even more MDBs?" They do show that some of the $CD73^{-/-}$ mice fed DDC formed a lower amount of high molecular weight (HMW) proteins and ubiquitinated HMW proteins (their fig. 4D). The normal K8 blot, on the other hand, was greatly increased by DDC feeding in the $CD73^{-/-}$ mice. This did not occur in the wild-type mice where MDBs were formed. These paradoxical observations are hard to reconcile. One explanation could be that low levels of CD73 somehow causes a decrease in 26S proteasome activity. However, the absence of CD73 does the opposite, that is, it prevents the loss of 26s proteasome activity. MDBs form when a shift occurs in the formation of the 26s proteasome. This shift is to the formation of the immunoproteasome.[2,3] The shift to form the immunoproteasome is the result of the response of interferon (IFN) sequence response element on the UbD (FAT10 in humans) promoter stimulated by IFNγ and tumor necrosis factor alpha (TNFα).[4] UbD is markedly induced in the DDC mouse model of MBD formation.[5] SAMe prevents this DDC induction of UbD, MDB formation, and the induction of immunoproteasome formation.[3,5] $UbD^{-/-}$ mice fed DDC cannot form MDBs.[6]

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View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.26953
Potential conflict of interest: Nothing to report.

Reply:

We thank Dr. French for his comments and questions about our study on the role of the ecto-nucleotidase CD73 in mouse liver Mallory-Denk body (MDB) formation.[1] CD73 is expressed in multiple tissues, including the lung, heart, brain, kidney, liver, and the immune system.[2] Although formation of extracellular adenosine is considered its primary function, CD73 also may act in a number of other ways independent of its enzymatic role.[3]

As Dr. French pointed out, our findings that $CD73^{-/-}$ mice are resistant to MDB formation is somewhat counterintuitive, given our initial observation that the MDB-susceptible mouse strain (C57BL/6J) is characterized by having lower CD73 mRNA and protein levels under basal conditions compared to the MDB-resistant C3H/He strain. However, we also showed that after 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) treatment there was a near complete loss of cell surface CD73 (AMPase) activity, but only a 60% decrease in overall CD73 activity (measured biochemically after tissue lysis). This observation, together with our findings of DDC-induced loss of plasma membrane CD73 in isolated hepatocytes, illustrates the complex nature of CD73 regulation in this model of liver injury. Therefore, the subcellular distribution (organellar versus plasma membrane) and regulation (such as glycosylation changes and enzymatic activity) of CD73 during hepatocellular injury are likely a more important determinant of hepatocyte injury than the overall CD73 expression levels.

Furthermore, the MDB resistance in $CD73^{-/-}$ mice also may be immune-related, given the known major role of CD73 in regulating inflammatory and immune responses.[4] For example, in absence of CD73 to catalyze the final and irreversible step in the conversion of extracellular adenosine triphosphate (ATP) to adenosine, the ATP levels in the vicinity of damaged and dying cells may significantly increase. This can accelerate the clearance of damaged cells by promoting phagocytosis by way of a number of mechanisms.[5] Altered purinergic signaling on other immune cell

types, such as regulatory T cells,[6] also may alter the course of liver injury progression in CD73$^{-/-}$ mice.

In summary, resistance to MDB formation in CD73$^{-/-}$ mice likely is related to a number of hepatocyte-intrinsic and -extrinsic mechanisms. Generation of mice with targeted deletion of CD73 from specific cell types should help resolve many outstanding questions pertaining to the functions of this enzyme in the liver.

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View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.26952
Potential conflict of interest: Nothing to report.

Vitamin D Levels in Patients With Chronic Hepatitis B

To the Editor:

We read with interest the article by Farnik et al.[1] regarding the low vitamin D levels in patients with chronic hepatitis B virus (HBV). A remarkable finding was the inverse relationship between serum vitamin D levels and HBV viral load.

A previous study in patients with chronic hepatitis C had also shown that necroinflammatory activity as well fibrosis scores were independently linked to low vitamin D levels.[2] Because of small cohort size, the researchers could not show an association between serum vitamin D levels and fibrosis scores. However, considering that necroinflammatory activity was not included in statistical analysis in this study; we would like to point out that high necroinflammatory activity may also explain the low vitamin D levels in patients with HBV. Before drawing a definitive conclusion, more details ought to be presented about this important issue.

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View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.26962
Potential conflict of interest: Nothing to report.

Trends in Hepatitis C Treatment Uptake in the United States

To the Editor:

In May 2011, the United States Food and Drug Administration (FDA) approved the direct-acting antiviral (DAA) drugs boceprevir (BOC) and teleprevir (TVR) to be used in combination with peginterferon and ribavirin (PEG/RBV) for genotype 1 (G1) chronic hepatitis C (HCV). These drugs offered improvements in efficacy and reduced duration for some patients, benefits somewhat compromised by increased adverse events and pill burden.

Major increases in treatment uptake rates are required to impact the growing burden of disease from HCV complications including cirrhosis and hepatocellular carcinoma.[1] However, it is not clear whether HCV treatment uptake increased since the availability of BOC and TVR. We surveyed prescription activity in the USA after the FDA approval of BOC and TVR, using proprietary databases (see online methods).

The impact of these medications on the total number of HCV patients treated was small (Table 1). Over 80,000 patients were

Table 1. Prescription Rates for Hepatitis C Therapy 2008-2012

Year	PEGalfa-2A	PEGalfa-2B	Telaprevir	Boceprevir
2008	57,544	26,081	N/A	N/A
2009	53,066	17,340	N/A	N/A
2010	53,610	13,311	N/A	N/A
2011	64,466	12,790	26,177	7,932
2012	52,656	11,596	36,801	14,787