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Sander Lefere[1,*]
Helena Degroote[1]
Hans Van Vlierberghe[1]
Lindsey Devisscher[2,*]
[1]*Department of Gastroenterology and Hepatology, Hepatology Research Unit, Ghent University, Ghent, Belgium*
[2]*Gut-Liver Immunopharmacology Unit, Basic and Applied Medical Sciences, Ghent University, Ghent, Belgium*
*Corresponding authors. Address: Gut-Liver Immunopharmacology Unit, Basic and Applied Medical Sciences, Ghent University, Corneel Heymanslaan 10, Building B, Entrance 36, B-9000 Ghent, Belgium. Tel.: +32 9 332 56 65.
E-mail addresses: sander.lefere@ugent.be
lindsey.devisscher@ugent.be

Reply to: "Unveiling the depletion of Kupffer cells in experimental hepatocarcinogenesis through liver macrophage subtype-specific markers"

"Who are you?", cell surface marker expression profiles of tissue-resident Kupffer cells and liver infiltrating myeloid cells

To the Editor:

During the last years, great efforts have been undertaken to uncover the origin and function of tissue-resident macrophages. Kupffer cells (KCs) are embryo-derived liver-resident macrophages, which are essential to combat liver infection, and which are critically involved in liver regeneration.[1–3] Recent data indicate that KCs have a distinct origin and function when compared with monocyte-derived macrophages. During homeostasis, KCs are self-maintained by local proliferation, with no additional input from bone marrow-derived cells.[1–4] However, upon activation of the immune system either by infection, carcinogenesis, or intoxication, the composition of the hepatic macrophage pool can change dramatically. A lively discussion about the fate of KCs after liver insult is currently ongoing, as highlighted by the comment of Lefere *et al.* in the current issue.[5] While we and others discovered a rapid KC loss upon infection,[6,7] this phenomenon was initially not detected in diethylnitrosamine (DEN)-induced hepatocellular carcinoma (HCC).[8] In contrast, KC loss was apparent in 2 other HCC models.[5] These observations led to the question of whether KC loss after liver injury is a general phenomenon, or whether the KC fate is dependent on the nature of the liver insult.[9,10]

Recently new surface markers suitable for classification of tissue-resident macrophages, including KC, were identified, which allow discrimination of local KCs and inflammatory macrophages.[4,11] One of these markers, Clec4F, was established as a specific KC marker, whereas Tim4 is expressed by a variety of long-lived tissue-resident macrophage subsets.[12–14] While, KC classification under homeostatic conditions is well established, we and others discovered that under inflammatory conditions KC classification is more challenging (Table 1).[4,6,8] Using the recently identified tissue-resident macrophage marker Tim4, Lefere *et al.* followed up on the question of whether the local KC pool is depleted upon acute acetaminophen (APAP) - induced liver injury and chronic inflammation. In accordance with previous data, they detected a profound KC loss during both, acute and chronic liver inflammation.[5,15] Additionally, they observed infiltration of monocyte-derived cells. Thus, in contrast to Kessler *et al.*, Lefere *et al.* concluded that KC loss is also present in the APAP model. Therefore, it is tempting to speculate that, as proposed earlier by Alazawi and Knolle,[9] KC loss is a general phenomenon during liver injury. The differences detected in the studies by Kessler *et al.* and Lefere *et al.* could have several explanations. Since it is rather likely that KC loss in the APAP model is transient, it is crucial to study KC disappearance at several time points after liver injury. While KC loss can be monitored by immunolabeling with the aforementioned cell surface markers Clec4F and Tim4, it is not possible to dissect the origin of reappearing KC with these markers, since upon liver infiltration their expression can also be upregulated on bone marrow-derived macrophages. Therefore, to address the origin of myeloid cells found in tissues, fate-mapping approaches are needed. Under acute inflammatory conditions, we and others used CX3CR1-GFP mice to dissect the origin of myeloid cell populations.[6,15] However, these mice are not suitable for studying chronic conditions, such as HCC. For the analysis of chronic inflammation, the use of inducible fate-mapping models such as CX3CR1-CreERT2 mice or a Cre-recombinase-driven barcoding system that generates tens of thousands of individual barcodes in a tissue- and time-controlled manner[16] could be suitable approaches. Scott *et al.* generated Clec4F-Cre mice which support KC-specific gene targeting.[17] This mouse will be instrumental in future studies specifically addressing KC functions.

Table 1. Myeloid cell composition after acute and during chronic liver injury.

Liver insult	KC loss	KC proliferation	Monocytes	MoKC	Reference
Acute					
Bacterial infection	+	+	+	+	7
Irradiation	+	+	+	+	5
Viral infection	+	n.d.	+	+	6
APAP treatment	+	+	+	?	15
Chronic					
DEN-induced HCC	n.d.	?	+	?	8
NASH	+	+	+	+	11
APAP treatment	+	?	+	?	5
DEN-induced HCC	+	?	+	?	5
NASH-induced HCC	+	?	+	?	5

? = unknown; n.d. = not detected; + = detected.
APAP, acetaminophen; DEN-induced HCC, diethylnitrosamine-induced HCC; HCC, hepatocellular carcinoma; KC, Kupffer cell; MoKC, monocyte-derived KC; NASH, non-alcoholic steatohepatitis.

Until recently, in the human system there were no specific markers available to distinguish between tissue-resident KCs and infiltrating macrophages. Nevertheless, a recent single-cell RNA sequencing study identified 2 distinct macrophage subpopulations in the human liver, anti-inflammatory KC and MARCO^{+} pro-inflammatory macrophages/monocytes.[18] This first classification will help to further characterize human macrophage populations and to study KC behavior during human liver inflammation.

Since KCs are sensors of tissue damage that may recruit inflammatory monocytes, one might interpret the KC loss as a self-inflicted brake of the immune system to prevent excessive inflammation. This hypothesis is supported by the observation that KC depletion attenuates the course of chronic inflammatory diseases such as NASH.[19,20] In contrast, tissue-resident macrophages were recently shown to inhibit inflammation after minor insults or during homeostasis.[21–23]

So far, in most studies it has not been addressed why and how KC loss is established. KC loss could be initiated by different types of cell death, depending on the stimulus and local cytokine milieu. Therefore, the different models could be associated with different types of KC loss. Importantly, KC can undergo apoptosis and necroptosis.[7] There are still many open questions regarding transient KC loss, including why KC disappear in many models of liver inflammation and how this KC loss is mediated. Finally, it will be critical to understand whether KC loss is an essential step to control liver inflammation or a consequence of the inflammation. Dissecting the different functions and characteristics of tissue-resident KC versus infiltrating monocytes is of utmost importance in order to better understand the biology of liver injury. Such knowledge might point towards new strategies to better treat hepatitis.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

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Katharina Borst[1,†]
Theresa Graalmann[2,3,†]
Ulrich Kalinke[2,4,*]
[1]*Institute of Neuropathology, Medical Faculty, University of Freiburg, Germany*
[2]*Institute for Experimental Infection Research, TWINCORE, Centre for Experimental and Clinical Infection Research, A Joint Venture between the Hanover Medical School and the Helmholtz Centre for Infection Research, Brunswick, Germany*
[3]*Clinic for Immunology and Rheumatology, Hannover Medical School, Hannover, Germany*
[4]*Cluster of Excellence – Resolving Infection Susceptibility (RESIST), Hannover Medical School, Hannover, Germany*
*Corresponding author. Address: Institute for Experimental Infection Research, TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany. Tel.: +49 511220027100.
E-mail address: kalinke.ulrich@mh-hannover.de

† Authors contributed equally.

Liver transplantation using hepatitis B core positive grafts: Which is the optimal antiviral prophylaxis?

To the Editor:

We read with great interest the article by Wong *et al.*[1] regarding the impact of hepatitis B core antibody (anti-HBc) positive liver grafts on survival and risk of hepatitis B (HBV) infection after liver transplantation (LT). The authors evaluated 964 LT recipients who received anti-HBc positive (n = 416, 43.2%) or anti-HBc negative (n = 548, 56.8%) liver grafts. Interestingly, donor anti-HBc status had no impact on long-term patient and graft survival, irrespective of graft peri-operative characteristics and recipient model for end-stage liver disease score at the time of LT. This finding is very encouraging in the era of liver graft shortages, particularly as it did not confirm the results of a previous study[2] in which the use of anti-HBc positive liver grafts was associated with worse post-LT outcomes.

The authors also evaluated the risk of *de novo* HBV infection after LT.[1] There were 108 HBV surface antigen (HBsAg)-negative (38 both anti-HBc/anti-HBs positive, 22 anti-HBc positive only, 24 anti-HBs positive only, 24 both anti-HBc/anti-HBs negative) recipients who received liver grafts from anti-HBc positive donors. Of them, 64 received lamivudine and 44 entecavir monoprophylaxis post-LT. *De novo* HBV infection, defined as post-LT detectable serum HBsAg and/or HBV DNA in HBsAg-negative recipients, was observed in 4.7% of patients under lamivudine (3/64) and none of the patients under entecavir (0/44), a numerical but not statistically significant difference ($p = 0.269$). Based on this finding, Wong *et al.*[1] suggested that antiviral agents with a high barrier to resistance (*i.e.* entecavir or tenofovir) should be used as first-line antiviral prophylaxis in HBsAg-negative recipients who receive anti-HBc positive liver grafts, in contrast to the current international guidelines which recommend lamivudine in this setting.[3] However, we believe that the authors' suggestion was based on findings which need further interpretation and should be considered with caution. First, all 3 patients diagnosed with *de novo* HBV infection had repeatedly undetectable HBV DNA and were only diagnosed based on HBsAg seropositivity. This HBV negative/HBsAg positive serological pattern is of unclear clinical significance in HBV-transplant recipients.[4] Second, HBsAg was reported to be only transiently positive in 2 of these patients who soon became HBsAg-negative and eventually developed anti-HBs. The third patient was found to be HBsAg positive at 1 month post-LT and presumably remained HBsAg positive (it is not clearly stated in the paper), but had undetectable serum HBV DNA on several occasions and no evidence of HBV-related histological lesions on 3 liver graft biopsies which were all negative for immunohistochemical staining for HBsAg and HBcAg. Third, the impact of the recipient's anti-HBc/anti-HBs combination status in relation to the type of HBV prophylaxis (lamivudine or entecavir) was not reported, although it has been shown to affect the probability of *de novo* HBV infection in this setting.[4] In particular, our previous systematic review[4] showed that the risk of *de novo* HBV infection in HBsAg-negative recipients who receive anti-HBc positive liver grafts but no antiviral prophylaxis after LT is just 1.4% in recipients positive for both anti-HBc and anti-HBs, but as high as 13.1% or 9.7% in those positive for only anti-HBc or anti-HBs. In agreement with the latter findings, the authors reported that none of the 38 recipients positive for both anti-HBc and anti-HBs developed

Keywords: Hepatitis B core antibody; Liver transplant; Hepatitis B; Hepatitis B core antibody positive graft; *De novo* hepatitis B infection.