To the Editor:

In a recent issue of this journal, Stewart et al. [1] presented a major breakthrough in liver research models. The authors used transgenic mice expressing herpes simplex virus thymidine kinase (TK) under the glial fibrillary acidic protein (GFAP) promoter. Proliferation of hepatic stellate cells (HSC) was first induced by CCL4 and then mice were treated with ganciclovir, which was metabolized by TK into toxic nucleotide analogues, ultimately leading to HSC death (≈70% depletion). Afterwards, mice were subjected to ischemia/reperfusion and to endotoxin treatments. The role of HSC in these acute injuries was studied – so far it has not yet been evaluated in detail. Notably, the magnitude of liver disease was lowered in HSC depleted mice, as expressed by reduced serum transaminases, neutrophil infiltration, tumor necrosis factor-α (TNF-α), and endothelin-A receptor in both conditions. In contrast, levels of interleukin-6 and endothelin-1 increased significantly, but only in the ischemia/reperfusion and endotoxin treatment, respectively [1]. Endothelin-B receptor behaved differently, being unchanged (ischemia/reperfusion) or as much elevated in depleted and non-depleted mice after endotoxin treatment. The authors concluded that HSC should be major producers of TNF-α, neutrophil chemoattractant factors, and endothelin-A receptor, thus having a critical role in ischemia/reperfusion and in endotoxin induced liver injuries.

Throughout years, a leading role has been ascribed to Kupffer cells (KC) in acute liver conditions [2]. They may have been dethroned, but it is noteworthy that a recent review published in this journal, devoted to KC heterogeneity, emphasized the crosstalk between HSC and KC, with mutual activation among these cells [3]. In this vein, we may wonder if the depletion of HSC could drive KC into a less inflammatory phenotype.

In recent years, we have been committed to the quantitative study of liver, establishing that HSC and KC comprise 6.4 and 8.9% of liver cells, respectively [4]. More recently, we used optical dissectors (20 μm height), that grant unbiased estimation of number [5], and concluded that 42 ± 7% of HSC were vicinal to KC (Fig. 1). It should be stressed that such juxtaposition only stands out in thick sections encompassing the size of both cells (even so, some appear in Supplementary Fig. 4 of Stewart et al. [1]). Besides paracrine stimuli, such juxtaposition should favor the crosstalk and mutual activation between cells, contacting through fenestrae of sinusoidal endothelial cells. It is well established that co-culture with KC or their conditioned medium stimulates the activation of HSC [3,6] and some functions, e.g., selective killing of HSC, only occur when juxtaposition with KC takes place [7]. Nowadays it is recognized that different subsets of KC coexist in the liver [3,8,9] and a recent study showed that HSC conditioned medium favored the differentiation of pro-inflammatory macrophages [9]. Stewart et al. [1] reported that the depletion treatment had no effect on KC, but we may hypothesize that the loss of HSC-KC juxtaposition (and of paracrine stimuli by HSC) could promote a switch of KC activation, lowering their inflammatory response. Hypothetically, a CD68+ KC phenotype could emerge, attracting less neutrophils and producing less TNF-α [8]. Considering that KC predominantly express endothelin-B receptors [10], this would also justify the puzzling pattern of endothelin-A vs. B receptors. In conclusion, the KC phenotype shift hypothesis would partially explain the data presented by Stewart et al. [1], being alternative (or complementary) to the view of HSC as having a critical role in acute liver conditions. This hypothesis deserves further attention from future studies using the HSC depletion method in acute liver injuries.

The whole liver may be viewed as an orchestra, with well-defined string, woodwind, and brass instruments (hepatocytes, KC, and HSC in this metaphor). If in vitro studies produce simpler tunes (one or two instruments), the methodology presented by Stewart et al. [1] creates a much more complex and appealing symphony. However, we are only starting to hear the melody, and still not know if all brass instruments are alike, which are missing and how the orchestra will hold without them.

Conflict of interest

The author declares that he does not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Depletion of hepatic stellate cells: Have Kupffer cells lost their bad neighbor?

Fig. 1. Thick liver section immune-stained against GFAP and CD163 for detecting hepatic stellate cells (HSC) and Kupffer cells (KC), respectively. The liver of 5 male Wistar rats (2 months) was collected and 5 thick paraffin sections (35 μm), per animal, were used to quantify the juxtaposition of HSC-KC. After antigen retrieval (4 + 4 + 4 minutes in the microwave), a double immunohistochemistry was performed against GFAP (rabbit polyclonal (Dako) 1:1500, 4 days) and CD163 (mouse monoclonal (Serotec) 1:100, another 4 days). Cells were systematic uniform random sampled and counted in optical dissectors (minimum of 200 cells per animal, sorting apart and juxtapositioned cells). HSC (diaminobenzidine brown color, open arrow) and KC (aminophenylcarbazole red color, block arrow) were counted using a counting frame of 1673 μm² (here depicted for illustrative purposes). Cells were counted if their nucleus was in focus and inside the inclusion (green) lines, not touching the exclusion (red) ones; bar = 6 μm. (This figure appears in colour on the web.)
Reply to: “Depletion of hepatic stellate cells: Have Kupffer cells lost their bad neighbor?”

To the Editor:
We appreciate the insightful commentary by Marcos on our recently published manuscript [1], where they suggest that the loss of communication between hepatic stellate cells (HSCs) and closely associated Kupffer cells can be an important mechanism of the amelioration of ischemia/reperfusion (I/R)- or LPS-induced injury in HSC-depleted liver. We agree with this view since Kupffer cells have been shown to elicit inflammatory response, and their blockade or elimination ameliorates hepatic injury in several animal models [2]. The role of Kupffer cells in mediating HSC activation and activation-dependent fibrogenic activity through mediators including free radicals, TNF-α and TGF-β is well documented [3]. However, little is known about the influence of HSCs on Kupffer cells in physiology and pathology. Thus based on the observations of our report, the view that juxtacrine (physical contact) and paracrine (via soluble mediators) interactions between these two cell types, due to their close proximity, regulate physiologic and pathophysiologic processes in the liver is a rational assessment.

The interactions between Kupffer cells and HSCs, however, must be considered to be very complex due to heterogeneity in the individual cell populations. Evidence suggests that the M1 Kupffer cells play a key role in inflammatory diseases whereas M2 Kupffer cells promote resolution of inflammation and tissue injury [4]. Furthermore, heterogeneity in Kupffer cells is also demonstrated with their differential phagocytic activity and expression of biologically active mediators in periportal vs. pericentral regions [5,6]. Similarly, heterogeneity between periporal and pericentral HSCs has also been illustrated [7]. Nevertheless, our results [1] indicate that HSCs elicit pro-inflammatory response in Kupffer cells based on the reduced expression of TNF-α and CXCL1, and increased or unaltered expression of IL-6 in stellate cell-depleted mice after I/R or LPS challenge. We also observed a modest increase in anti-inflammatory cytokine IL-10 expression in HSC-depleted compared with control liver in both models of hepatic injury (unpublished finding). Since HSCs themselves produce all of these mediators [8,9], their interactions with Kupffer cells become even more interesting and important in hepatic inflammation and injury.

The property of HSCs to influence the characteristics of other cell types may not be limited only to Kupffer cells. For example, greater increase in endothelin-1 expression upon LPS challenge of HSC-depleted liver compared to the control liver [1] indicates their regulation of hepatic endothelial cells, the primary cell type to produce this vasoconstrictor peptide [10]. Furthermore, HSCs were shown to induce tolerance to myeloid dendritic cells and increase immunosuppressive potential of regulatory T cells via physical contact, and these interactions were also found to alter pro- and anti-inflammatory cytokine production by either cell type [8,9].

We, however, note that in our study both Kupffer cells and HSCs should be considered quiescent when the mice were subjected to I/R or endotoxin challenge [1]. Although we do not exclude the possibility that HSC depletion may already have altered Kupffer cell characteristics, the mode of interactions between them is expected to be quite different with their activation during progression of chronic liver injury. Thus, the hypothesis that depletion of HSCs drives Kupffer cells to less inflammatory phenotype (M1 to M2 switch) proposed by Marcos and Lopez will be an interesting area for future investigation both in acute and chronic liver injury.

REFERENCES


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