

Reply to: “Dendritic cell subset composition in the human liver is more complex than it seems”

To the Editor:

We read with interest the letter from Strauss and Bartlett regarding our study of Dendritic Cells (DCs) in healthy human liver [1] and would like to develop several of the points they raise. CD141 is a type 1 membrane receptor that binds thrombin and is normally expressed by endothelial cells [2]. Its role on the surface of immune cells remains a puzzle and the functional relevance of variable expression by subsets of DCs is not known. DCs obtained from some organs, e.g., the skin, express CD141 on both CD14[−] and CD14⁺ subsets of antigen presenting cells [3]. However, the majority of liver DCs expressing CD141 are CD11c⁺ and CD14[−] [1,3]. In addition, neither CD123⁺ plasmacytoid DCs nor CD1c⁺ myeloid DC subsets appear to express high levels of CD141 in the liver [1]. Extensive functional analyses of these subsets are required before the puzzle can be solved, a particularly challenging task given the small numbers of cells and difficulty of getting healthy human tissue.

For functional characterisation of hepatic CD141⁺ DCs we used a magnetic bead isolation method which was chosen in order to maximize yield and limit activation or contamination due to flow sorting, as did Lauterbach *et al.*, 2010 [4] for their demonstration of IFN- λ production from blood CD141⁺ DCs. To increase purity, we ran our samples over two columns as per manufacturer instructions, resulting in cell populations that were highly enriched for CD141⁺ cells. (High CD141 expressing DCs are clearly distinguishable from low CD141 expressors in the immuno-fluorescence image in Fig. 2A, which is of a mixed population of liver immune cells.)

Since we began this work, the CLEC9a marker was established as a useful marker of DC subsets when used together with CD141 and we found that the CD141 high population of DCs in the liver also co-expressed CLEC9a. Future work on this population of cells would be best served by isolation of double positive CD141⁺CLEC9a⁺ cells. Complete characterisation of the entire antigen-presenting cell repertoire of the liver may well require addition of even more immune cell markers.

Use of liver perfusate as a source of liver resident APCs certainly has its limitations. We may well be studying cell populations that have higher migratory properties than others. We observe lower frequencies of iNKT cells in liver perfusate (unpublished data) compared to what we [5] and others have found in liver biopsies, suggesting that this particular population is less migratory than conventional NK cells. However there is still no ideal method for the study of minor cell subsets from healthy human liver tissue. Too many markers are required to accurately define DC subsets to use immunohistochemistry. Digestion of tissue prior to flow cytometry may also skew results, if, as previously demonstrated [6], certain epitopes are sensitive to

digestion enzymes. Also rare cell subsets may be lost in the multiple washing steps required in such methods. DCs are at too low frequency to study in liver biopsies and they are unlikely to be normal if obtained from tumour bearing liver tissue as significant differences in iNKT, and monocyte populations as well as cytokine levels [7–9] have been found in liver tissue from patients undergoing resection when compared with healthy liver. Moreover, it has recently been demonstrated that tumour microenvironment profoundly suppresses DC function [10]. Perhaps DC subset distribution and function in healthy liver tissue may be better explored using alternative methods in centres where adequate tissue might be obtained, e.g., during resizing of liver for paediatric transplantation.

It is clear that the CD141⁺ population of DCs is heterogeneous as evidenced by differing expression of ILT3 and ILT4 [1]. The presence of these subsets in both healthy and diseased livers warrants further study. While some liver resident DCs may be tolerogenic, our work clearly illustrates the ability of CD141⁺ DCs from healthy human liver to secrete inflammatory cytokines and drive T cell responses. These proinflammatory liver resident APCs are good targets for development of vaccine adjuvants or immunotherapy.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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