Editorial for J Hep paper: "Functions of human liver CD69+CD103- CD8 T cells depend on HIF2a activity in healthy and pathological livers"

Liver-resident CD8$^+$ T cells: learning lessons from the local experts

L. Swadling*[1]

L.J. Pallett*[1]

M.K. Maini[1,2]

[1] Division of Infection & Immunity, Institute of Immunity & Transplantation, University College London, UK

[2] Corresponding Author: M. K. M. (m.maini@ucl.ac.uk); ORCID: 0000-0001-6384-1462; UCL Infection and Immunity, Rayne Institute, 5 University Street, London WC1E 6JF

*These authors contributed equally

Recent studies in humans as well as animal models have highlighted the importance of CD8$^+$ memory T cells compartmentalised at the site of disease, known as tissue-resident memory T cells (CD8$^+$ T_{RM}). These local immune sentinels are highly adapted to maintain functionality within tissues, providing efficient *in situ* immunity at hotspots for pathogen encounter. They can provide continual immunosurveillance and pathogen control by the rapid production of critical mediators such as IFNγ [1]. Conversely, CD8$^+$ T_{RM} have been linked with tissue-specific damage and autoimmunity [2]. It is clear then, that T_{RM} in general can be considered "friend," whilst maintaining successful immune control, or "foe", when contributing to immunopathology or autoimmunity.

But what is known about CD8$^+$ T_{RM} in the human liver? Tissue-resident CD8$^+$ T cells (including hepatitis B virus [HBV]-specific responses) have been previously identified in the human liver, with increased frequencies associated with better control of HBV infection [3–6]. However, more than two decades ago, evidence emerged supporting a dual role for CD8$^+$ T cells in the HBV-infected liver, with HBV-specific CD8$^+$ T cells that failed to control viral replication exacerbating progressive organ damage via the recruitment of bystander non-antigen-specific cells [7–9]. This concept was elegantly extended in a recent publication by Kim *et al* showing that bystander liver damage in viral hepatitis can be mediated by innate-like cytolytic activity of CD8$^+$ T cells [10]. In this edition of Journal of Hepatology, Kim *et al* accessed large number of perfusates from *living* liver donors to investigate the heterogeneity within the CD8$^+$ T_{RM} compartment to further unpick their roles in immunity and

pathology (Kim *et al* J hep 2020). Their data suggest that a subset of those liver $CD8^+$ T cells that are capable contributing to bystander liver damage can be regulated by hypoxia-inducible factor (HIF)-2α.

Two markers consistently associated with $CD8^+$ T_{RM} are the tissue retention molecules; CD69 (S1PIR antagonist, preventing tissue egress) and CD103 (integrin αE that binds E-cadherin [3,6]); however, phenotypic and functional diversity that is yet to be decoded exists within T_{RM} subsets defined using these markers alone [1,2,11](**Table 1**). The human liver houses sizeable populations of both $CD69^+CD103^-$ and $CD69^+CD103^+$ $CD8^+$ T cells, that can be separated from infiltrating, but non-resident, $CD69^-CD103^-$ T cells by their expression of several features common to resident memory T cells ($S1PR1^{hi}$, CXCR6, CD49α and a lack of KLF2 expression Kim *et al* J hep 2020 [1–3,6]). Kim *et al* specifically highlight the numerical dominance of the $CD69^+CD103^-$ T_{RM}-like population, that express chemokine receptors supporting liver retention (CXCR6, CXCR3, and a lack of CX_3CR1 [1,3,6]) but exhibit an intermediate phenotype between the non-resident, tissue-infiltrating $CD69^-CD103^-$ and $CD69^+CD103^+$ T_{RM}. Prolonged exposure to environmental cues may be needed for a $CD69^+CD103^-$ T_{RM}-like cell to differentiate into a double positive $CD69^+CD103^+$ T_{RM}; cytokines, such as TGFβ and IL33, are known inducers of CD103 expression [6,12]. TCR engagement by cognate antigen recognition within the tissue may also drive CD103 expression [6,13]: in support of this, Kim *et al* find more CD103 expression on intrahepatic T cells specific for hepatotropic compared to non-hepatotropic viral infections (Kim *et al* J hep 2020).

The $CD69^+CD103^-$ T_{RM}-like population described by Kim *et al* are enriched for terminally differentiated memory T cells expressing CD45RA (T_{EMRA}; CD45RA re-expression, $CCR7^-$), which are also readily identified in the blood, and expand with age and CMV seropositivity [14]. Despite their name, T_{EMRA} are far from inert or fully senescent T cells as they have the ability to mediate pathogen clearance and/or tissue damage via innate-like cytotoxic pathways, such as NKG2D [14]. Interestingly, Kim *et al* show that $CD69^+CD103^-$ T_{RM}-like cells responded to stimulation with the prototypical liver cytokine IL-15 by upregulating NKG2D, which also conferred on them innate-like cytolytic function, in line with the authors' recent study ([10]; Kim *et al* J hep 2020). Whilst IL-15-driven expansion of heterologous $CD8^+$ T cell responses may contribute to protection in acute viral infections [15], in situations of chronic inflammation it can represent a tissue-specific danger signal, that promotes damage through licensing of bystander T cells and NK cells [16]. The highly abundant $CD69^+CD103^-$ T_{RM}-like population could, therefore, represent a pool of T cells lodged within the local microenvironment with the potential to mediate cytotoxic bystander tissue damage.

The liver has regions that are hypoxic, attributable to its dominant venous blood supply and sluggish sinusoidal blood flow [17]. T cells can sense the local oxygen gradient and adjust their gene expression by stabilising the transcription factors HIF1α and HIF2α [18]. Thus, the expression of HIF2α by $CD69^{+}CD103^{-}$ T_{RM}-like cells suggests they may be located in particularly hypoxic regions, although this remains to be investigated. Using pharmacological or genetic knock-down of HIF2α, Kim *et al* show that this transcription factor is required by $CD69^{+}CD103^{-}$ T cells for survival and optimal functionality upon bystander or TCR-stimulation (Kim *et al* J hep 2020). Although HIF1α expression has been linked to T cell differentiation and metabolic reprogramming [19], the role of HIF2α has not been studied in detailed. $CD69^{+}CD103^{-}$ T cells are the dominant population in livers during acute hepatitis A infection and cirrhosis, and their expression of HIF2α was further increased in disease (Kim *et al* J hep 2020). Potential links between hypoxia and HIF-dependent T cell functionality, and their roles as biomarkers and drivers of liver pathology require further study.

HIF2α was uniquely enriched within intrahepatic $CD69^{+}CD103^{-}$ T cells compared to T_{RM} from other human tissues such as, lung, colon and kidney. It is unsurprising that T cells manifest organ-specific adaptations considering that each site is anatomically distinct and encounters different microbial and pathogenic insults. For example, T cells can respond to arginine starvation by increasing nutrient transporters for the uptake of alternative amino acids (CD98 [20]), an adaptation noted on human liver-resident T cells [6]. Another adaptation recently discovered in human T cells that reside in the hostile, hypoxic liver environment is an increase in basal autophagy levels, a process that can remove the depolarised mitochondria that limit metabolic flexibility of exhausted T cells, as well as providing biomolecules for cellular metabolism [21]. Conversely, murine liver T_{RM} are enriched for the purinergic receptor P2RX7, allowing them to be selectively depleted by ischemia and other causes of sterile tissue damage such as acetaminophen poisoning [22].

Future research efforts should aim to address the many unresolved questions regarding the functionality of heterogenous T_{RM} subsets in the liver, to pave the way for the development of novel vaccines and immunotherapies tackling the global health priorities targeting the liver, including hepatitis B and C infection, malaria, and hepatocellular carcinoma. A pressing issue to address is the *in situ* localisation of T_{RM} subsets and their relevant interactions with the underlying parenchyma and other cell types. The liver has a complex structure with distinct anatomic regions where different cellular, soluble, and stromal mediators could influence T cell migration and function, which cannot be adequately assessed with traditional methods for dissociating bulk

tissues for single cell analysis. High resolution *in situ* multiparameter imaging of well-preserved tissue sections will further reveal functional and phenotypic differences dependent on topological features, whilst the state-of-the-art technique NICHE-seq, combining photoactivatable reporters and singe-cell RNA-sequencing [23], could allow detailed spatial reconstruction of the liver-resident T cell niche. The existence of *bona fide* resident populations mediating hepatic immunosurveillance has been shown for murine $CD8^+$ T cells by parabiosis experiments [11]; the visualisation of these T_{RM} localised within the liver sinusoidal vasculature is compatible with the observation, in this and previous studies, that they can be isolated from liver perfusates. Whilst $CD69^+CD103^+$-expressing T cells are exclusively found in the liver, the $CD69^+CD103^-$ T cell fraction in the liver (the focus of Kim *et al* 's study) has partially overlapping features with some circulating populations (summarised in **Table I**). Thus, additional phenotypic and/or transcriptional markers that can better distinguish the full complement of subsets that are exclusively liver-resident are still needed. In addition, further studies confirming the longevity and retention of $CD8^+$ T cells with a T_{RM} phenotype in the human liver would support their therapeutic potential in providing sustained local immunosurveillance. Such immunosurveillance may extend to primary and secondary liver tumours, since emerging data support a critical role for $CD8^+$ T_{RM} in the immune control of tumours [24]. The capacity of liver T_{RM} to sense and drive tissue damage should also stimulate studies of their role in regulating liver inflammation and fibrosis in disease settings like non-alcoholic steatohepatitis. The novel finding by Kim *et al* of HIF2α-dependent intrahepatic T cells underscores the value of sampling liver-resident immune populations in studies of pathological tissues and therapeutic interventions (whether by biopsy or fine needle aspiration [4])(Kim *et al* J hep 2020), to learn more lessons from the "local experts". 1449 words

	Marker (gene name)	Blood non-Temra	Blood Temra	$CD69^-$ $CD103^-$ Liver infiltrating	$CD69^+$ $CD103^-$ T_{RM}-like	$CD69^+$ $CD103^+$ T_{RM}
	Frequency in the liver	na	na	++	++/+++	+/++
Tissue retention	CD69	+ *	+ *	-	++	+++
	CD103 (*ITGAE*)	-	-	-	-	++
	CXCR3	-	-	-	++	+++
	CXCR6	-	-	-	++	++
	CD49a (*ITGA1*)	*?*	*?*	-	+	++
	CX_3CR1	+	+	+	-	-
	SIPR1	+++	*?*	+++	-	-
	LFA-1 (*ITGAL*)	+	+	+	+++	++
Transcription Factors	Blimp1 (*PRDM1*)	-	-	-	++	+++
	Hobit (*ZNF683*)	++	*?*	++	-	-
	Tbet (*TBX21*)	++	+++	++	++	+
	Eomesodermin (*EOMES*)	+	+++	+	+++	+
	TCF1	++	-	*?*	*?*	*?*
	KLF2	++	++	++	-	-
	HIF2α	+	*?*	+	++	+
Innate-like function / Senescence	NKG2D	-	++	-	++	++
	CD57 (*B3GAT1*)	-	++	++	++	+
	KLRG-1	-	+++	-	++	-
Differentiation / Specificity	CD45RA+ CCR7+, naïve	+++	-	+	-	-
	CD45RA- CCR7+, Tcm	+	-	-	-	-
	CD45RA- CCR7- , Tem	++	-	++	++	+++
	CD45RA+ CCR7- , Temra ^	-	+++	-	+	+/-
	PD-1 (*PDCD1*)	+	+	++	+++	+++
	HBV (hepatotropic virus)	+	+	+	+	+++
	CMV (non-hepatotropic virus)	++	+++	++	++	-

Table 1: Comparison of human blood and liver-resident $CD8^+$ T cell populations: The relative expression (from minimal - to highest +++) of markers on T cell subsets at the protein level. * CD69 expression on a small number of $CD8^+$ T cells in the blood has been attributed to recent activation. ^ $CD45RA^+$ are excluded from analysis of T_{RM} subsets in some publications. CMV, cytomegalovirus; HBV, hepatitis B virus; Tcm, central memory T cells; Tem, effector memory T cells, Temra, terminally differentiated effector memory; T_{RM}, tissue-resident memory T cell.

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