Editorial for J. Hep.

"IL-2 contributes to cirrhosis-associated immune dysfunction by impairing follicular T helper cells in advanced cirrhosis" – Basho *et al*

IL-2 leaves its mark in cirrhosis

A.R. Burton[1]
L.J. Pallett[1]

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

Corresponding Author: L.J.P. (laura.pallett@ucl.ac.uk); ORCID: 0000-0002-4161-9462; UCL Infection and Immunity, Rayne Building, 5 University Street, London, WC1E 6JF

Number of figures and tables: 1

Conflict of interest statement: *The authors have nothing to disclose*

Authors' contributions: ARB and LJP wrote this editorial

Financial support: ARB is supported by a Wellcome Trust Senior Investigator Award (101849/Z/13/A) & an NIHR-EME grant to Prof. Mala Maini; LJP is supported by a Wellcome Trust Senior Investigator Award (101849/Z/13/A) & CRUK Immunology grant (26603) to Prof. Mala Maini.

Keywords: cirrhosis; follicular helper T cells, IL-2, hypergammaglobulinemia; liver immunology

Cirrhosis is the leading cause of liver-related deaths worldwide and as such represents a significant cause of morbidity and mortality each year [1]. Although the disease burden and underlying aetiology varies by demographic group and location, the impact of chronic liver injury and/or inflammation results in the deposition of extracellular matrix proteins, excessive scarring, liver stiffening and the eventual loss of hepatocyte architecture and function [2]. Beyond the clear risks associated with liver failure and hepatocellular carcinoma, individuals with clinically decompensated cirrhosis are often also functionally immunosuppressed, as evidenced by a predisposition to bacterial infections and impaired responses to prophylactic vaccination [3].

Effective, durable humoral immune responses are generated in germinal centre (GC) reactions, whereby specialised populations of T follicular helper cells (T_{FH}) direct the differentiation and selection of high affinity B cell clones. Although functionally immunosuppressed, cirrhotic patients often present with raised levels of immunoglobulins in their blood (referred to as hypergammaglobulinemia [HGG]) – an observation at odds with their failure to mount protective immune responses. In this edition of Journal of Hepatology, Basho *et al* addressed this discrepancy by profiling circulating T_{FH} (cT_{FH}) responses in more than 100 patients with advanced cirrhosis [4]. Through thorough phenotypic assessment of the circulating $CD4^+$ T cell compartment, Basho *et al* have revealed a reduction in PD-1^+CXCR5^+ cT_{FH} that clonally and transcriptionally resemble GC-associated T_{FH} [5,6], in individuals with decompensated cirrhosis compared to those with compensated disease or non-cirrhotic controls. Interestingly, remaining cT_{FH} in decompensated individuals display a 'phenotypic imprint' indicative of enhanced interleukin 2 (IL-2) signalling, a feature well associated with the impairment of T_{FH} function and differentiation [7]. This IL-2 imprint on cT_{FH} is evidenced by elevated expression of the α-chain of the high affinity IL-2 receptor, CD25, and the co-stimulatory molecule, OX40 (**Fig.1**). Likewise, individuals presenting with decompensated cirrhosis have increased serum concentrations of soluble-CD25 (a surrogate marker associated with IL-2-induced immune activation), rendering $CD4^+$ T cells in these patients more sensitive to IL-2 signalling. In further support of IL-2 imprinting, cT_{FH} in decompensated individuals express less TCF1 - a transcription factor upstream of Bcl-6 that is inhibited by IL-2 (**Fig.1**). Since TCF1 is required for both the initiation of T_{FH} differentiation and for the maintenance of effector function in fully-differentiated T_{FH} [8,9], downregulation of its expression may play a critical role in impairing T_{FH} cell function. While this study revealed some clear changes to cT_{FH} responses in decompensated cirrhotic individuals, some phenotypic and functional T_{FH}-associated features remained the same. cT_{FH} expression of inducible T cell costimulatory (ICOS) and IL-21 secretion was maintained at similar levels between compensated and decompensated cirrhotic patients. As both support the capacity of T_{FH} to provide help to B cells, it could be suggested that T_{FH}-driven activation of B cell immunity is at least in part maintained in some patients with advanced cirrhosis.

In line with this, one intriguing aspect of this study is the observed association between cT_{FH} phenotype and the presence or absence of HGG. Here, Basho *et al* present data suggesting that patients with HGG (defined as a serum IgG titre > 1600 mg/dl) tend to have higher numbers of cT_{FH}, although this difference was not statistically significant. Crucially, cT_{FH} in patients with HGG exhibit a less pronounced IL-2 imprint, as evidenced by a less severe reduction in TCF-1 expression and decreased expression of CD25 on the surface of the cT_{FH} when compared to patients without HGG (**Fig.1**). Reduced IL-2 imprinting in patients with advanced cirrhosis presenting with HGG was associated with maintenance of a T_{FH} response, with cT_{FH} frequency and

function associated with serum titres of class-switched antibody and reduced risk of mortality. Interestingly, co-culture of activated T_{FH} cells and naïve B cells, in the presence of increasing concentrations of IL-2, resulted in down-regulation of the T_{FH}-lineage defining receptor CXCR5 and significant impairments to B cell differentiation, alongside a decrease in the concentration of secreted IgG and IgM. Combined, these data provide convincing evidence to suggest that the absence of HGG in patients with liver cirrhosis is associated with severe T_{FH} dysfunction and impaired humoral immunity, imparted by IL-2.

Although class-switched memory B cells were not altered between patient cohorts, the frequency of $CD27^+IgM^+IgD^+$ memory B cells was decreased in line with an altered frequency and function of T_{FH}. Beyond the requirement for T help, B cell changes may be influenced by other mechanisms, for example those directly imparted by endotoxemia and the increased translocation of gut bacterial antigens associated with cirrhosis. While the GC has remained the focus of B cell research, it has long been appreciated that antibody responses can also develop outside of the B cell follicle where GC responses occur. Extrafollicular differentiation is initiated following B cell antigen encounter, after which committed B cells proliferate and differentiate in extrafollicular foci, generating rapid and robust immunity. Activation of the extrafollicular pathway can be dependent or independent of T cell help, with both routes able to generate both memory and long-lived plasma cell responses [10] and associated with large antibody-secreting cell expansion. Importantly, extrafollicular responses can be initiated through a combination of IFNγ, IL-21 and toll-like receptor stimulation, and potentiated by a population of IgD^-CD27^- B cells [11]. Similar to findings in patients with advanced cirrhosis, we have previously identified an expansion of dysfunctional $CD27^-CD21^-$ atypical memory B cells (that phenotypically overlap with IgD^-CD27^- cells) within the intrahepatic B cell compartment relative to the blood [12], implicating the tolerogenic liver niche in driving this phenotype and impairing B cell immunity. Hence, in patients with decompensated cirrhosis, continuous stimulation by gut bacterial antigens may be driving extrafollicular B cell expansion, contributing to the increased frequencies of IgD^-CD27^- memory B cells and plasma cells observed in patients with decompensated cirrhosis.

This study identified a negative role for an IL-2 imprint on cT_{FH} in cirrhosis by impairing their differentiation and function, raising the possibility that T_{FH}-targeted blockade of IL-2 may have therapeutic potential in preventing immunosuppression in advanced cirrhosis. However, it is worth reflecting on the potential beneficial roles for IL-2 on other immune populations in patients with decompensated cirrhosis (with or without HGG) that are still to be explored. As the authors themselves point out, an increase in local IL-2 is likely to impact the overall survival of the antigen-specific T cell compartment by enhancing expression of the anti-apoptotic molecules (such as Bcl2). Similarly, IL-2 has long been shown to promote the proliferation and differentiation of naïve T cells into effector and memory T cells crucial for the success of adaptive immune responses. Importantly, IL-2 can overcome tolerance and rescue $CD8^+$ T cells rendered dysfunctional by priming in the liver [13,14]. Not addressed in this cohort of individuals with decompensated cirrhosis is the source of IL-2 or soluble-CD25, nor what triggers their production. It is tempting to speculate that these molecules are produced by immune cells, such as profibrogenic $CD14^+CD16^+$ mononuclear phagocytes previously shown to correlate with soluble-CD25 levels in the serum of patients with cirrhosis [15]. These cells may be activated by the presence of bacterial LPS in the portal circulation, arising as a consequence of increased cirrhosis-driven gut permeability. Equally

plausible sources of IL-2 are populations of activated $CD4^+$ T cells or liver-resident $CD8^+$ T cells sequestered in the liver [16], a postulate first proposed by Müller *et al* over 30 years ago [17].

In demonstrating that IL-2 leaves its mark by impairing by T_{FH} responses in patients with advanced cirrhosis, this work raises the possibility that either the number or phenotype of cT_{FH} could be used as a biomarker associated with survival in patients, reminiscent of recent data revealing that the phenotypic profile of cT_{FH} has predictive value when determining potential response rates to an immunotherapy in type 1 diabetes [18]. Likewise, increased IgG levels and the presence of HGG may be able to predict preserved T_{FH} function, reflecting an early stage of decompensation where the immune system is still able to respond to foreign antigens. With future studies it would be interesting to follow up whether patients with HGG are more able to mount protective responses to vaccination and have overall less profound functional immunosuppression attributable to maintained T_{FH} function.

Word Count: 1353

Figure Legend

An IL-2 imprint in advanced cirrhosis: the impact on the circulating T follicular (cT_{FH}) response. Basho *et al* reveal that the number of cT_{FH} are reduced in patients with decompensated cirrhosis, compared to compensated disease or in the absence of cirrhosis (control individuals). cT_{FH} in decompensated cirrhosis have elevated expression of the high affinity IL-2 receptor (CD25) and OX40, alongside a reduction in TCF-1. In the patients with hypergammaglobulinemia (HGG) the remaining cT_{FH} exhibit a less severe reduction in TCF-1 and decreased CD25 expression, potentially associated with a maintained T_{FH} response with implications for their susceptibility to bacterial infection and responses to prophylactic vaccination. Created with BioRender.

Word Count including figure legend: 1465

References:

[1] Roth GA, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet 2018;392:1736–88. https://doi.org/10.1016/S0140-6736(18)32203-7.

[2] Sepanlou SG, Safiri S, Bisignano C, Ikuta KS, Merat S, Saberifiroozi M, et al. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet Gastroenterology & Hepatology 2020;5:245–66. https://doi.org/10.1016/S2468-1253(19)30349-8.

[3] Albillos A, Lario M, Álvarez-Mon M. Cirrhosis-associated immune dysfunction: Distinctive features and clinical relevance. Journal of Hepatology 2014;61:1385–96. https://doi.org/10.1016/j.jhep.2014.08.010.

[4] Basho K, Zoldan K, Schultheiss M, Bettinger D, Globig A-M, Bengsch B, et al. IL-2 contributes to cirrhosis-associated immune dysfunction by impairing follicular T helper cells in advanced cirrhosis. Journal of Hepatology 2020;0. https://doi.org/10.1016/j.jhep.2020.10.012.

[5] Locci M, Havenar-Daughton C, Landais E, Wu J, Kroenke MA, Arlehamn CL, et al. Human Circulating PD-1+CXCR3−CXCR5+ Memory Tfh Cells Are Highly Functional and Correlate with Broadly Neutralizing HIV Antibody Responses. Immunity 2013;39:758–69. https://doi.org/10.1016/j.immuni.2013.08.031.

[6] Vella LA, Buggert M, Manne S, Herati RS, Sayin I, Kuri-Cervantes L, et al. T follicular helper cells in human efferent lymph retain lymphoid characteristics. J Clin Invest n.d.;129:3185–200. https://doi.org/10.1172/JCI125628.

[7] Ballesteros-Tato A, León B, Graf BA, Moquin A, Adams PS, Lund FE, et al. Interleukin-2 inhibits germinal center formation by limiting T follicular helper cell differentiation. Immunity 2012;36:847–56. https://doi.org/10.1016/j.immuni.2012.02.012.

[8] Xu L, Cao Y, Xie Z, Huang Q, Bai Q, Yang X, et al. The transcription factor TCF-1 initiates the differentiation of TFH cells during acute viral infection. Nat Immunol 2015;16:991–9. https://doi.org/10.1038/ni.3229.

[9] Wu T, Shin HM, Moseman EA, Ji Y, Huang B, Harly C, et al. TCF1 Is Required for the T Follicular Helper Cell Response to Viral Infection. Cell Reports 2015;12:2099–110. https://doi.org/10.1016/j.celrep.2015.08.049.

[10] Di Niro R, Lee S-J, Vander Heiden JA, Elsner RA, Trivedi N, Bannock JM, et al. Salmonella Infection Drives Promiscuous B Cell Activation Followed by Extrafollicular Affinity Maturation. Immunity 2015;43:120–31. https://doi.org/10.1016/j.immuni.2015.06.013.

[11] Jenks SA, Cashman KS, Zumaquero E, Marigorta UM, Patel AV, Wang X, et al. Distinct Effector B Cells Induced by Unregulated Toll-like Receptor 7 Contribute to Pathogenic Responses in Systemic Lupus Erythematosus. Immunity 2018;49:725-739.e6. https://doi.org/10.1016/j.immuni.2018.08.015.

[12] Burton AR, Pallett LJ, McCoy LE, Suveizdyte K, Amin OE, Swadling L, et al. Circulating and intrahepatic antiviral B cells are defective in hepatitis B. J Clin Invest 2018. https://doi.org/10.1172/JCI121960.

[13] Schurich A, Berg M, Stabenow D, Böttcher J, Kern M, Schild H-J, et al. Dynamic Regulation of CD8 T Cell Tolerance Induction by Liver Sinusoidal Endothelial Cells. The Journal of Immunology 2010;184:4107–14. https://doi.org/10.4049/jimmunol.0902580.

[14] Bénéchet AP, De Simone G, Di Lucia P, Cilenti F, Barbiera G, Le Bert N, et al. Dynamics and genomic landscape of CD8+ T cells undergoing hepatic priming. Nature 2019;574:200–5. https://doi.org/10.1038/s41586-019-1620-6.

[15] Seidler S, Zimmermann HW, Weiskirchen R, Trautwein C, Tacke F. Elevated circulating soluble interleukin-2 receptor in patients with chronic liver diseases is associated with non-classical monocytes. BMC Gastroenterology 2012;12:38. https://doi.org/10.1186/1471-230X-12-38.

[16] Pallett LJ, Davies J, Colbeck EJ, Robertson F, Hansi N, Easom NJW, et al. IL-2high tissue-resident T cells in the human liver: Sentinels for hepatotropic infection. J Exp Med 2017;214:1567–80. https://doi.org/10.1084/jem.20162115.

[17] Müller C, Knoflach P, Zielinski CC. Soluble interleukin 2 receptor in acute viral hepatitis and chronic liver disease. Hepatology 1989;10:928–32. https://doi.org/10.1002/hep.1840100606.

[18] Edner NM, Heuts F, Thomas N, Wang CJ, Petersone L, Kenefeck R, et al. Follicular helper T cell profiles predict response to costimulation blockade in type 1 diabetes. Nature Immunology 2020;21:1244–55. https://doi.org/10.1038/s41590-020-0744-z.