

# Novel targets in the immune microenvironment of the hepatic sinusoids for treating liver diseases

Patten, Daniel A; Shepherd, Emma L; Weston, Christopher J; Shetty, Shishir

DOI:

[10.1055/s-0039-1678727](https://doi.org/10.1055/s-0039-1678727)

License:

None: All rights reserved

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Patten, DA, Shepherd, EL, Weston, CJ & Shetty, S 2019, 'Novel targets in the immune microenvironment of the hepatic sinusoids for treating liver diseases', *Seminars in Liver Disease*, vol. 39, no. 2, pp. 111-123.  
<https://doi.org/10.1055/s-0039-1678727>

[Link to publication on Research at Birmingham portal](#)

## **Publisher Rights Statement:**

Checked for eligibility: 03/05/2019

Copyright: Thieme Medical Publishers

<https://www.thieme-connect.de/products/ejournals/abstract/10.1055/s-0039-1678727>

## **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

## **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

**Novel targets in the immune microenvironment of the hepatic sinusoids for treating liver diseases**

Corresponding Author

Dr Shishir Shetty MBChB PhD FRCP, Centre for Liver Research, 5<sup>TH</sup> Floor IBR, Institute of Immunology and Immunotherapy, Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT

Email [s.shetty@bham.ac.uk](mailto:s.shetty@bham.ac.uk)

Tel +441214158700

Fax +441214158701

Co-Authors

Dr Daniel A Patten PhD, Centre for Liver Research, 5<sup>TH</sup> Floor IBR, Institute of Immunology and Immunotherapy, Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT

Dr Emma L Shepherd PhD, Centre for Liver Research, 5<sup>TH</sup> Floor IBR, Institute of Immunology and Immunotherapy, Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT

Dr Christopher Weston PhD, Centre for Liver Research, 5<sup>TH</sup> Floor IBR, Institute of Immunology and Immunotherapy, Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT

26

## 27 **Abstract**

28 Immune dysregulation and accumulation of leukocytes is a hallmark of adult chronic  
29 liver diseases. Progressive hepatic inflammation can lead to fibrosis and cirrhosis  
30 with a high risk of liver failure or hepatocellular cancer (HCC). Recent advances  
31 have been made in the treatment of liver disease including the development of highly  
32 effective antiviral therapy for hepatitis C and the potential of immunotherapy for  
33 HCC. Despite this, the majority of other chronic liver diseases including alcoholic  
34 liver disease, fatty liver disease and cholestatic diseases do not respond to  
35 conventional anti-inflammatory therapies. Recent studies defining the organ-specific  
36 properties that contribute to resident immune activation and immune cell recruitment  
37 from the circulation in these conditions have identified novel hepatic inflammatory  
38 pathways which are now being targeted in clinical trials. Further understanding of  
39 how the immune microenvironment is regulated within the liver and how disease  
40 specific mechanisms alter this process will hopefully lead to combination therapies to  
41 prevent aberrant inflammation and also promote fibrosis resolution. In this review,  
42 we focus on the advances that have been made in identifying key components of the  
43 inflammatory pathway including the recognition of danger signals, the recruitment  
44 and retention of lymphocytes from the circulation and the pathways which promote  
45 resolution.

46

## 47 **Main Concepts and Learning Points**

- 48 1. The majority of adult chronic liver diseases are driven by inflammatory  
49 processes which are unresponsive to conventional anti-inflammatory  
50 therapies.

2. Recent work has highlighted the major role of macrophages, tissue resident Kupffer cells and recruited monocytes, in sensing hepatic damage which drives downstream immune responses.
3. Lymphocyte recruitment via the hepatic sinusoids contributes to hepatitis and is mediated by interactions with liver sinusoidal endothelial cells via typical and atypical adhesion molecules.
4. Clinical trials are targeting macrophage responses to epithelial damage and immune cell recruitment via adhesion molecules as novel anti-inflammatory approaches in chronic liver disease.
5. Further approaches to treat hepatic inflammation should take into account inflammatory pathways which mediate immune cell retention in liver tissue and promote resolution of fibrogenesis.

Adult inflammatory liver diseases lead to a major global burden on human health, and patients with progressive disease are at risk of developing fibrosis and cirrhosis which can culminate in end-stage liver failure or hepatocellular cancer (HCC), both of which are associated with extremely high mortalities<sup>1</sup>. Recent advances have been made in the treatment of liver disease, especially in the field of viral hepatitis. The development of direct-acting antivirals for the treatment of hepatitis C has demonstrated very high rates of viral eradication<sup>2</sup>. In the case of hepatitis B, current therapies are effective at suppressing viral replication and can reduce necroinflammation with reversal of fibrosis as well as reducing HCC risk<sup>3,4</sup>. In contrast, the inflammatory processes that drive other major liver diseases such as alcoholic liver disease, non-alcoholic steatohepatitis and cholangiopathies have

continued to be a major therapeutic challenge. For those patients who progress to advanced chronic liver disease there are limited options when they develop end-stage liver disease, with transplantation being the only choice in many cases<sup>5</sup>. New therapies are therefore urgently required to reduce the burden on transplantation and the associated high waiting list mortality.

Adult chronic liver diseases are driven by inflammation, which promotes epithelial damage and death leading to the activation of resident immune cells and the accumulation of circulating immune cells recruited from the circulation<sup>6,7</sup>. Each disease has a specific pattern of injury which is dependent on the site of initial damage. For example, NASH is triggered by hepatocyte damage characterised by sublethal injury associated with lipotoxicity, resulting in parenchymal inflammation associated with innate and adaptive immune responses<sup>8</sup>. In contrast, primary sclerosing cholangitis is driven by cholangiocyte injury leading to the localised release of chemokines and pro-inflammatory cytokines associated with portal inflammation and ductal proliferation and ductular loss<sup>9</sup>. These inflammatory processes are associated with the activation of hepatic stellate cells and if left unchecked lead to excessive deposition of extracellular matrix, fibrosis and persistent damage culminating in cirrhosis<sup>10</sup>. The site of injury determines the pattern of fibrosis with parenchymal diseases such as ALD/NASH presenting centrilobular and sinusoidal fibrosis and cholangiopathies associated with periportal fibrosis leading to irregular shaped nodules<sup>11</sup>.

Targeting the inflammatory pathways that drive these conditions has the potential of inhibiting fibrogenesis, but the mechanisms involved are poorly understood. Autoimmune hepatitis for example is often responsive to steroid-based therapy and

immunomodulators, whereas other immune-mediated liver diseases such as primary sclerosing cholangitis and primary biliary cholangitis are currently unresponsive to these medications<sup>9,12</sup>. Furthermore, patients suffering from the major inflammatory liver diseases secondary to alcohol and non-alcoholic fatty liver disease do not derive benefit from current anti-inflammatory approaches. We therefore urgently require better understanding of the underlying core inflammatory pathways that drive these diseases to identify novel therapies which can prevent the progression to cirrhosis and end stage liver disease.

In this review, we focus on three major processes which are implicated in chronic inflammatory liver diseases, the immune response to danger signals released by persistent epithelial damage, the recruitment/retention of immune cells from the circulation and the factors which drive resolution and repair within the liver.

### **The immune response to danger signals released from epithelial damage.**

Epithelial damage is a key factor in initiating inflammatory liver diseases. This involves cellular stress secondary to factors such as lipotoxicity in fatty liver disease, accumulation of breakdown products of alcohol and hepatotropic viruses. These processes are associated with the release of danger signals or danger associated molecular patterns (DAMPs) into the microenvironment. How these danger signals are sensed and processed by the innate immune system is one of the key determinants of progression of these inflammatory conditions<sup>13</sup> (summarised in Figure 1).

#### *Kupffer cell recognition of DAMPS*

126 The major cellular population to sense and respond to these danger signals are the  
127 liver resident macrophages, Kupffer cells. Kupffer cells are the sentinels of the liver  
128 and are derived from yolk sac precursors which self renew<sup>14</sup>. They play a role in  
129 processing gut-derived products and mediating immune responses to microbes.  
130 Additionally, they sense sterile injury and associated DAMPS which are a  
131 characteristic of the major inflammatory liver diseases including alcoholic  
132 steatohepatitis (ASH) and non-alcoholic steatohepatitis (NASH). DAMPs which  
133 have been associated with Kupffer cell activation include high mobility group protein  
134 B1 (HMGB1), ATP, uric acid, DNA fragments and cholesterol crystals<sup>14</sup>. Targeting  
135 the pathway of DAMP recognition is already underway in clinical trials. DAMPs are  
136 recognised by pattern recognition receptors including Toll-like receptors (TLRs) and  
137 scavenger receptors which are both highly expressed by macrophages. TLR4 has  
138 been studied extensively and a TLR4 antagonist, JKB-122, is currently undergoing  
139 assessment in the setting of NASH as an early phase II clinical trial NCT02442687.  
140 Galectin-3 expressed on Kupffer cells which is a member of the scavenger receptor  
141 family which recognise the terminal galactose residues on glycoproteins. Galectin-3  
142 plays a key role in hepatic uptake of advanced lipoxidation and glycation end  
143 products<sup>15</sup>. An agent which binds galectin-3, GR-MD-02, is progressing through  
144 early stage clinical trials in the setting of NASH<sup>16</sup>. Other members of the scavenger  
145 receptor family which have been implicated in promoting hepatic inflammation  
146 include CD36 and Scavenger Receptor-A (SR-A). Targeted deletion of these  
147 receptors on myeloid cells, led to reduced levels of inflammation and fibrosis in  
148 models of fatty liver disease<sup>17</sup>. A recent study confirmed that the recognition of  
149 DAMPs, specifically products of lipid peroxidation such as malondialdehyde (MDA)-  
150 LDL, by CD36 and SR-A led to the release of pro-inflammatory cytokines<sup>18</sup>. Blocking

the action of these receptors may therefore be beneficial in the setting of NASH. Interestingly, the authors also targeted the DAMP directly, in this case the MDA epitope, by *in vivo* neutralization with antibodies. This approach was successful in reducing inflammation in their pre-clinical model of fatty liver disease.

#### *The role of the inflammasome in chronic liver disease*

Whilst the direct recognition of DAMPs is a viable pathway to target, there are also downstream pathways which play significant roles in the progression of chronic liver disease. The recognition of these danger associated ligands by pattern recognition receptors on Kupffer cells leads to the formation of the inflammasome. Inflammasomes are multi-protein complexes which are comprised of a nucleotide oligomerization domain (NOD)-like receptors and effector molecules including pro-caspase-1, and adaptor molecules e.g. apoptosis-associated speck-like CARD-domain containing protein (ASC)<sup>19</sup>. Following the formation of the inflammasome, Kupffer cells produce inflammatory mediators, such as interleukin 1beta and other pro-inflammatory cytokines and chemokines. Activation of inflammasome complexes have been confirmed in pre-clinical models of alcoholic liver injury and fatty liver disease<sup>20</sup>. This leads to the recruitment of other innate populations from the circulation such as neutrophils, monocytes and populations of T cells. Therefore targeting the pathway of inflammasome formation is also a rational approach to prevent progression of inflammatory liver diseases. Studies in pre-clinical models of alcoholic liver disease demonstrated that targeting the inflammasome pathway by pharmacological inhibition of IL-1R1 prevented the development and progression of alcoholic liver disease<sup>21</sup>. Additionally, one the most extensively studied inflammasomes in macrophages is the NOD-, LRR- and pyrin domain-containing 3



(NLRP3) inflammasome which has previously been shown to play a critical role in the progression of murine models of non-alcoholic fatty liver disease<sup>22</sup>. A recent study confirmed its role in driving liver inflammation and fibrogenesis by studying liver injury in mice with constitutive activation of NLRP3 in myeloid cells. Activation of the NLRP3 inflammasome led to excess production of TNF and IL-17 resulting in severe inflammation and fibrosis<sup>23</sup>.

### *Recruitment of peripheral monocyte populations*

Another major downstream consequence of Kupffer cell driven inflammation is the recruitment of other monocyte populations from the circulation via the CCL2-CCR2 axis<sup>24,25</sup>. The chemokine CCL2 promotes recruitment of CCR2<sup>+</sup> monocytes from the circulation, and this has been confirmed in experimental models of both alcoholic liver disease and fatty liver disease<sup>26</sup>. A recent study confirmed the increased accumulation of CCR2<sup>+</sup> macrophages within liver tissue parallels with fibrosis progression in fatty liver disease. These populations of cells were seen as aggregates of monocyte-derived macrophages around portal tracts<sup>27</sup>. Furthermore, gene analysis of these recruited (monocyte-derived macrophages) versus resident (Kupffer cells) confirmed that monocyte-derived macrophages were associated with multiple growth factors and cytokines leading to fibrosis progression, whereas Kupffer cells were characterised by factors associated with inflammation initiation. Therapeutic targeting of the recruitment of these CCR2<sup>+</sup> monocytes by administration of Cenicriviroc a CCR2/CCR5 dual chemokine receptor antagonist led to amelioration of hepatic inflammation and fibrosis in several models of NASH<sup>27</sup>. In keeping with these findings, there is encouraging clinical experience that Cenicriviroc could be a potential therapy for chronic liver disease. A phase 2b study of this agent

in patients with non-alcoholic steatohepatitis and established fibrosis demonstrated a significant improvement in fibrosis compared to placebo after 1 year of treatment<sup>28</sup>. Activated Kupffer cells also secrete several other chemokines including CCL25, CX3CL1, CXCL2 and CXCL8<sup>14</sup>; thus, targeting these chemokines may also influence the recruitment of other distinct immune populations from the circulation during inflammatory liver disease leading to other novel targets for treatment. An intriguing recent study has also identified the recruitment of immune populations from the peritoneal compartment. In a model of sterile liver injury a population of GATA6-positive macrophages were detected at a very early stage of tissue damage. These GATA6<sup>+</sup> macrophages migrated directly across the mesothelium and their recruitment was dependent on the adhesion molecule CD44 and adenosine triphosphate<sup>29</sup>. The role of these macrophages in the progression of chronic inflammatory liver diseases and their therapeutic potential is yet to be confirmed.

#### *The activation of unconventional lymphocytes*

In parallel to the initiation of inflammation by myeloid populations, there is gathering interest in the role of unconventional lymphocytes which are found highly enriched in epithelial tissues and have well established roles in anti-microbial immunity<sup>30</sup>. Their roles in early immune responses has led investigators to study if they could be pivotal in the triggering and regulation of progressive liver disease.  $\gamma\delta$  T cells are predominantly generated in the thymus and characterised by a  $\gamma\delta$  T cell receptor (TCR), they only account for 2-3% of all CD3<sup>+</sup> T cells in secondary lymphoid organs but have been found to be enriched in the liver<sup>31</sup>.  $\gamma\delta$  T cells recognise conserved structures including non-peptide metabolites and heat shock proteins. They can rapidly release cytokines which are known to regulate adaptive immune populations

226 including conventional  $\alpha\beta$  T cells and therefore have been postulated as an  
227 additional link between innate and adaptive immune responses<sup>32</sup>. Experimental  
228 models of liver disease have demonstrated the accumulation of these cells during  
229 liver injury and their contribution to disease progression. In a murine model of  
230 autoimmune hepatitis,  $\gamma\delta$  T cells played a protective role associated with reduced  
231 liver damage and inflammatory cytokine levels. In this setting the protective  
232 mechanism was found to be regulated by IL-17 produced by  $\gamma\delta$  T cells  
233 downregulated the function of another family of unconventional T cells, natural killer  
234 T (NKT) cells<sup>33</sup>. Further support for the protective role of these cells in liver disease  
235 has been demonstrated in models of chronic liver injury. Murine models of fibrosis  
236 and steatohepatitis demonstrated that the CCR6<sup>+</sup> subset of  $\gamma\delta$  T cells prevented  
237 fibrosis by promoting the apoptosis of hepatic stellate cells<sup>34</sup>.

238 As alluded to earlier, another subset of unconventional lymphocytes, NKT cells,  
239 appear to promote inflammatory liver disease. NKT cells are lymphocyte subsets  
240 which express cell surface markers associated with NK cells as well as the T cell  
241 receptor and they are characterised by their recognition of glycolipid antigens. They  
242 have been shown to localise to the hepatic sinusoids and demonstrate a  
243 crawling/patrolling phenotype<sup>35</sup>. NKT cells accumulated in models of liver injury and  
244 were shown to promote hepatic inflammation and contributed to progressive  
245 fibrosis<sup>36</sup>. Further studies focused on the potential contribution of NKT cells to fatty  
246 liver disease. Higher levels of NKT cells were detected in patients undergoing  
247 transplantation for NASH compared to other indications, this accumulation was also  
248 seen in murine models of NASH and mice deficient in NKT cells were protected from  
249 fibrosis in this model<sup>37</sup>. Subsequent studies implicated hepatic NKT cells in the

250 increased production pro-fibrogenic factors including osteopontin and hedgehog  
251 ligands<sup>38</sup>.

252 Further understanding of the functional properties of another unique subset of  
253 innate-like T cells, mucosal-associated invariant T cells (MAIT) cells, has highlighted  
254 their potential as regulators of liver inflammation. MAIT cells are characterised by  
255 the expression of a semivariant TCR that recognises a MHC-like protein (MR-1)<sup>39</sup>.  
256 MR-1 presents vitamin B metabolites derived from commensal and pathogenic  
257 bacteria and thus MAIT cells can be activated by a variety of bacterial strains<sup>40</sup>. The  
258 high levels of these cells in human gut biopsies and accumulation in lamina propria  
259 led to them being named MAIT cells<sup>41</sup>. Subsequent studies have now shown that  
260 they are also enriched in the liver and have explored their antimicrobial properties in  
261 immune mediated liver disease and alcoholic liver disease<sup>42-44</sup>. This has led  
262 investigators to speculate that MAIT cells may make a significant immune  
263 contribution in the liver acting as a firewall between the host and gut derived  
264 bacteria<sup>45</sup>. However these reports have also shown that MAIT cells are highly  
265 activated in the liver and are the predominant IL-17 producers within the hepatic T  
266 cell compartment and could therefore be important drivers of aberrant hepatic  
267 inflammation. A recent study has studied the contribution of these cells in chronic  
268 liver injury. MAIT cells were found to be enriched in the periportal region and along  
269 the fibrotic septa in tissue from cirrhotic livers and in a carbon tetrachloride model of  
270 chronic liver injury these cells were found to be pro-fibrogenic by promoting the pro-  
271 inflammatory properties of both monocyte-derived macrophages and fibroblasts<sup>46</sup>.

272 Unconventional lymphocytes are therefore a novel target to treat chronic  
273 inflammatory liver disease, but further work is clearly required to understand how to  
274 either manipulate their function or utilise them as cell therapy.

## **Lymphocyte recruitment via the liver sinusoids**

The accumulation of adaptive immune cell populations within the liver is also a hallmark and driver of all adult chronic inflammatory liver diseases. A prerequisite for leukocyte recruitment from the circulation into organs is their interaction with endothelial cells lining blood vessels. In general, leukocyte migration from the blood into inflamed tissues occurs in post-capillary venules<sup>47</sup>; however, in the liver, this process occurs in the low shear flow microvasculature of the hepatic sinusoids which are lined by liver sinusoidal endothelial cells (LSEC)<sup>7</sup> (Figure 2). LSECs are a phenotypically and functionally unique population of endothelial cells. They are characterised by a minimal basement membrane and atypical cellular junctions as well as membranous pores organised in sieve plates called fenestrations<sup>48</sup>. Additionally, LSECs are also characterised by the expression of an array of scavenger receptors (SRs)<sup>49</sup>. These structural and phenotypic characteristics support the physiological functions of LSEC but they also influence the mechanisms of lymphocyte recruitment and thus are potential organ specific anti-inflammatory targets. The low shear stress environment of the hepatic sinusoids negates the requirement for the early rolling steps of the leukocyte adhesion cascade<sup>7</sup>. As a consequence, LSEC express negligible levels of selectins<sup>50</sup>, a small family of transmembrane  $\text{Ca}^{2+}$ -dependent lectins which play an integral role in the initial stages of leukocyte recruitment in more conventional vascular beds<sup>51</sup>. A critical step in determining if lymphocytes accumulate at sites of inflammation is not only their adhesion to endothelium but also their subsequent transmigration across the

endothelial barrier. We now know that the process of transendothelial migration (TEM) in itself is a multi-step pathway involving a combination of receptor interactions which are potential therapeutic targets for inflammation<sup>52</sup>. The conventional route for TEM by leukocytes is via the paracellular route (in between cells, through cellular junctions), but it has also been shown that leukocytes can migrate via the transcellular route (directly through the endothelial body)<sup>53</sup>. Studies on human LSEC demonstrate that a significant proportion of lymphocytes migrate via the transcellular route<sup>54</sup>. Additional *in vitro* studies, demonstrated that the structure of these endothelial cells permits a novel migratory pattern, where lymphocytes were shown to migrate directly into LSEC and then migrate into adjacent endothelial cells<sup>55</sup>. This migration was dependent on interferon gamma and facilitated by the unique junctional complexes between LSEC. This work highlights that the sinusoidal vascular bed is not a simple barrier but plays an active role in regulating the immune microenvironment within the liver and the positioning of lymphocytes in liver tissue. Further work has elucidated some the molecular contributors to this process and their potential as novel anti-inflammatory targets.

#### *Conventional adhesion molecules*

Several studies have demonstrated that LSEC use a unique combination of both conventional endothelial adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), and atypical adhesion molecules to mediate lymphocyte recruitment in chronic liver disease<sup>56,57</sup>. VCAM-1 binds the leukocyte-expressed  $\alpha_4\beta_1$  integrin<sup>58</sup> and plays an important role in capturing lymphocytes from blood flow within the hepatic sinusoids and subsequently mediates stabilisation<sup>59,60</sup>. ICAM-1 supports firm adhesion of

lymphocytes, via binding to  $\alpha_L\beta_2$  integrin (lymphocyte function-associated molecule-1 (LFA-1))<sup>61</sup>, and subsequently mediates their transmigration across LSEC<sup>54,62</sup>. Both VCAM-1 and ICAM-1 are significantly upregulated by proinflammatory factors, such as cytokines<sup>63</sup>; however, their adhesive function is largely dependent on the formation of endothelial adhesive platforms (EAPs)<sup>64</sup>. EAPs play an essential role in the spatial organisation of VCAM-1 and ICAM-1 within the cell membrane, resulting in concentrated areas of expression of the adhesion molecules in the contact area with adherent leukocytes<sup>64</sup>. The formation of EAPs has been proposed to be regulated by the tetraspanin family of receptors, which are able to laterally associate with adhesion molecules to form microdomains<sup>64,65</sup>. In support of this previous work, the tetraspanin CD151 associated with VCAM-1 within LSECs and was able to regulate lymphocyte adhesion under physiological flow conditions *in vitro*<sup>66</sup>. Due to their widespread constitutive expression in a number of cell types and tissues, VCAM-1 and ICAM-1 are unlikely to represent viable therapeutic targets; however, modulating their lateral interactions with tetraspanins, such as CD151, may present an attractive and organ-specific target for chronic inflammatory liver disease.

Mucosal vascular addressin cell adhesion molecule-1 (MAdCAM-1), which belongs to the immunoglobulin family along with VCAM-1 and ICAM-1, is known to bind to the  $\alpha_4\beta_7$  integrin<sup>67</sup> and plays an important role in lymphocyte trafficking to the gut, via mucosal vessels<sup>68</sup>. Under normal physiological conditions, MAdCAM-1 is absent from the liver; however, previous studies have demonstrated that MAdCAM-1 can be upregulated through the enzymatic activity of an atypical adhesion molecule, vascular adhesion protein-1 (VAP-1), in LSEC in some chronic liver diseases<sup>69</sup>. This is particularly evident in primary sclerosing cholangitis (PSC), where it promotes the

recruitment of, gut-activated T cells which express high levels of  $\alpha_4\beta_7$  integrin<sup>70,71</sup>. Its hepatic functionality is highly supportive of immunological crosstalk between the gut and the liver, and MAdCAM-1 might contribute to the pathophysiological link between inflammatory bowel disease (IBD) and PSC, a progressive autoimmune biliary disease which is associated with IBD in ~80% of cases. Currently, clinical trials are being considered to target MAdCAM-1/ $\alpha_4\beta_7$  interactions in PSC using therapeutic antibodies originally developed for the treatment of IBD. Trials have included a selective humanised monoclonal antibody, Vedolizumab, to  $\alpha_4\beta_7$ . Prior clinical studies with Vedolizumab in the setting of IBD have confirmed that this drug can modulate lymphocyte recruitment to the gut in both ulcerative colitis and Crohn's disease leading to a reduction in inflammation and improved mucosal healing<sup>72,73</sup>. This has led to gathering interest in the use of Vedolizumab in the setting of diseases where MAdCAM-1 has been shown to be upregulated, particularly PSC. Until recently, this had involved single centre case series with results suggesting safety and improvement of inflammatory parameters<sup>74</sup>. A multi-centre study has now been completed in patients with PSC and IBD which demonstrated clinical responses in the IBD pathology, and the drug was safely tolerated, but it did not lead to any detectable improvement in liver biochemistry<sup>75</sup>. Whether targeting the MAdCAM-1/ $\alpha_4\beta_7$  interaction could improve long term outcomes in PSC, including prevention of progressive fibrosis, transplant-free survival and cancer incidence, still needs to be addressed.

#### *Atypical adhesion molecules*

Vascular adhesion protein-1 (VAP-1) is a membrane-bound amine oxidase that, under normal physiological conditions, is expressed in vascular endothelial cells,



smooth muscle cells, and adipocytes<sup>76</sup>. During homeostasis VAP-1 is localised to cytoplasmic vesicles in endothelial cells, but under inflammatory conditions the protein is trafficked to the cell surface<sup>77</sup>. Early studies of VAP-1 showed that it mediated leukocyte binding to high endothelial venules (HEVs), the specialised post-capillary venules found in lymph nodes<sup>78</sup>. Further studies confirmed that VAP-1 was expressed at high levels in chronically diseased liver tissues *ex vivo*<sup>79</sup> and directly mediated adhesion and transmigration across LSEC *in vitro*<sup>80</sup>. In addition, via its enzyme activity, VAP-1 can upregulate expression of other adhesion molecules (e.g. VCAM-1, ICAM-1 and MAdCAM-1) and chemokines (e.g. CXCL8) in LSECs, consequently enhancing leukocyte recruitment<sup>69,81</sup>. More recently, these results have been corroborated *in vivo*, confirming the multifaceted role of VAP-1 in leukocyte recruitment to the liver in murine models of liver injury, and described VAP-1 expression by hepatic stromal cell populations<sup>82</sup>. A number of preclinical studies targeting VAP-1 have confirmed that inhibition of its enzymatic activity and/or blockade of its adhesive function with therapeutic antibodies reduces leukocyte infiltration in a range of rodent models of inflammatory diseases<sup>83</sup>.

Scavenger receptor that binds phosphatidylserine and oxidized lipids (SR-PSOX), which in its soluble form is also known as the chemokine, CXCL16, is expressed by LSEC<sup>84</sup> and is upregulated in both acutely<sup>85,86</sup> and chronically injured liver tissues<sup>87</sup>. CXCL16 is a specific ligand for the chemokine receptor CXCR6, thus enabling its membrane-bound form to interact with intrahepatic CXCR6<sup>+</sup> immune cells, such as effector T cells<sup>87,88</sup>, natural killer (NK) cells<sup>89,90</sup> and NKT cells<sup>84</sup>. Genetic deficiency of SR-PSOX has recently been shown to reduce the extent of inflammation and necrosis in a murine model of acetaminophen (APAP)-induced acute liver injury<sup>85</sup>.

400 Additionally, and perhaps more encouragingly, pharmacological intervention with  
401 neutralising antibodies against SR-PSOX has shown efficacy in reducing  
402 inflammation in preclinical murine models of sepsis-mediated<sup>86,91</sup> and carbon  
403 tetrachloride (CCl<sub>4</sub>)-mediated<sup>92</sup> acute liver injury. Furthermore, Wehr and colleagues  
404 were also able to demonstrate the efficacy of SR-PSOX antibody therapy in a  
405 commonly used murine model of non-alcoholic steatohepatitis (NASH), showing a  
406 reduction in both macrophage infiltration and triglyceride levels. Therefore, targeting  
407 the SR-PSOX (CXCL16)/CXCR6 axis may hold promising potential for treatment of  
408 inflammation and subsequent fibrosis of the liver<sup>92</sup>.

409  
410 The class H scavenger receptor stabilin-1, also known as common lymphatic  
411 endothelial and vascular endothelial cell receptor (CLEVER-1), was originally shown  
412 to mediate lymphocyte transmigration across HEVs<sup>93</sup>. Given the phenotypic  
413 similarities between lymphatic endothelial cells and LSEC<sup>50</sup>, stabilin-1 was found to  
414 be expressed in human liver and shown to be significantly upregulated in the hepatic  
415 sinusoids in chronic liver disease<sup>54</sup>. Following this, adhesion assays with lymphocyte  
416 subsets demonstrated that stabilin-1 specifically mediated transendothelial migration  
417 of T<sub>regs</sub> and B-cells through LSECs *in vitro*, under conditions which mimic the  
418 physiological flow and proinflammatory microenvironment of the hepatic sinusoids  
419 during liver injury<sup>54,62</sup>. This was the first demonstration of a T<sub>reg</sub>-specific adhesion  
420 molecule and transmigration of this lymphocyte subset was shown to be dependent  
421 on a combination of stabilin-1, VAP-1 and ICAM-1. T<sub>regs</sub> play a vital role in promoting  
422 tolerance, they mediate immunosuppression through multiple mechanisms and  
423 prevent autoimmunity and counteract inflammatory reactions mediated by the  
424 effector arm of the immune system<sup>94</sup>. Therefore, in the context of inflammatory liver

diseases approaches to upregulate stabilin-1 or promote the function of stabilin-1 could promote T<sub>reg</sub> accumulation as a strategy to prevent progressive hepatitis.

The expression of the stabilin-1 homologue, stabilin-2, has also been described in LSEC and was originally shown to act as a clearance receptor for hyaluronan from the blood<sup>95,96</sup>. Through a number of mutation experiments and antibody blockade studies *in vitro*, Jung *et al.* found that stabilin-2 was also able to mediate lymphocyte binding and identified the integrin  $\alpha_M\beta_2$  as the lymphocyte-expressed ligand<sup>97</sup>. They also determined that stabilin-2 predominantly acts in the firm adhesion step of the leukocyte adhesion cascade as its silencing, via shRNA, did not affect lymphocyte rolling or transendothelial migration, but was still able to significantly reduce the number of adherent cells<sup>97</sup>. To date, the study by Jung *et al.* remains the sole investigation of the role of stabilin-2 in leukocyte recruitment to LSEC. Further work is required to understand how the stabilin receptor family expressed on LSEC contribute to lymphocyte recruitment in preclinical models of inflammatory liver disease.

Scavenger receptor class F, member 1 (SCARF1 or SR-F1), also known as scavenger receptor expressed by endothelial cells (SREC-I), has also been shown to be expressed in both murine and human LSEC<sup>98,99</sup>. Recently, it has been shown that SCARF1 plays a role in the selective recruitment of CD4<sup>+</sup> T cells to human LSEC, under physiological shear stress conditions *in vitro*<sup>99</sup>. In this study, SCARF1 contributed to the firm adhesion step of the leukocyte adhesion cascade, with endothelial surface expression of SCARF1 observed in adhesive cup structures formed on the surface of the LSEC<sup>99</sup>. However, SCARF1 is an understudied

450 scavenger receptor<sup>100</sup> and more research into the extent of the contribution of  
451 SCARF1 in immune cell recruitment is required before it can be considered as a  
452 therapeutic target. Nevertheless, SRs including SCARF1 have been shown to be  
453 upregulated in several human inflammatory liver diseases and appear to accumulate  
454 at the interface between inflammation/fibrosis and correlate with fibrosis progression.

455

#### 456 *Chemokines*

457 Chemokines are an important component in the process of leucocyte recruitment  
458 and contribute to both firm of adhesion of leukocytes to endothelium and their  
459 subsequent migration across the endothelium. They are a family of small proteins  
460 which bind to G-protein coupled receptors on the leukocyte surface and induce  
461 conformational changes of integrins which triggers firm adhesion<sup>101</sup>. They are also  
462 found within intraendothelial vesicles and promote transendothelial migration<sup>102</sup>. We  
463 have already highlighted their role in monocyte and NK/NKT populations but they  
464 also play a significant role on lymphocyte recruitment within the sinusoids. The most  
465 extensively investigated are the inflammatory chemokines CXCL9-11 which bind to  
466 the receptor CXCR3 and have been shown to be upregulated in a range of liver  
467 diseases<sup>103-105</sup> and functionally they contribute to the transendothelial migration of  
468 lymphocytes across primary human HSEC<sup>103</sup>. Previous studies have also shown  
469 that chemokines contribute to the compartmentalisation of lymphocytes in liver  
470 diseases with the CXCR3 ligands promoting recruitment into the parenchyma  
471 whereas CCR5 ligands (the chemokines CCL3-5) contribute to portal tract  
472 recruitment<sup>103,106,107</sup>. The contribution of chemokines to inflammation provides a  
473 clear rationale for targeting them as novel anti-inflammatories but a recent study  
474 highlights the difficulties of achieving sustained inhibition of chemokines. NI-0801 is

a human monoclonal antibody against the CXCR3 ligand, CXCL10, which was studied in the context of PBC<sup>108</sup>. Investigators completed a phase 2a study in patients with PBC with inadequate response to ursodeoxycholic acid with the aim of assessing the safety and efficacy of NI-0801. The study demonstrated that the drug was safely tolerated and led to pharmacological responses in the blood but there was no therapeutic benefit identified with repeated infusions.

An alternative approach would be to consider targeting lymphocyte subsets, focusing on pro-inflammatory subsets and allowing persistent recruitment of regulatory subsets in order to shift the balance in the hepatic microenvironment. Whilst CXCR3 ligands have been implicated in the recruitment of several subsets including both T<sub>regs</sub> cells and subsets which secrete the pro-inflammatory cytokine IL-17 (Th17 cells)<sup>109,110</sup>, other chemokines were implicated in the subsequent migration into hepatic tissue of these subsets. T<sub>reg</sub> recruitment was regulated by the CCR4 ligands CCL17 and CCL22, whereas Th17 recruitment was regulated by CCL20, a CCR6 ligand<sup>109,110</sup>. In view of these findings, targeting the chemokine CCL20 rather than CXCR3 ligands may prove to be a more effective anti-inflammatory approach which will not alter T<sub>reg</sub> recruitment. Recent studies highlight the importance of the Th17/Treg balance in determining progressive inflammatory liver disease<sup>111-113</sup>.

#### **Retention of immune cells in the stromal compartment**

Following migration into the tissue, infiltrating immune cells are maintained in the local microenvironment. Complementary to the role of the endothelial layer, the stromal compartment of the liver maintains a microenvironment which permits the recruitment and retention of inflammatory cells. The hepatic stellate cell (HSC)

500 population are a hepatic stromal cell type which resides in a quiescent state in the  
501 sub-endothelial layer between the endothelium and the parenchymal cells, namely  
502 the space of Disse. Release of stimulating factors from injured epithelial cells and  
503 infiltrating immune causes the HSCs to become activated, driving a programme of  
504 proliferation, migration and contractility of HSC controlled by a plethora of both  
505 paracrine and autocrine stimuli. The consequence of this activation is the synthesis  
506 of extracellular matrix (ECM) proteins and subsequent accumulation of scar tissue.  
507 In view of the key role played by HSC in fibrogenesis, there has therefore been a  
508 vast drive to investigate how these cells may be targeted as a therapeutic strategy in  
509 liver disease (reviewed in <sup>114</sup>).

510

511 *In vitro* activated primary human HSCs and *in vivo* activated liver myofibroblasts  
512 (aLMFs) secrete a range of cytokines, chemokines and growth factors which can  
513 recruit and position leukocytes by G-coupled receptor-dependent and –independent  
514 mechanisms<sup>115</sup>. When cultured in basal conditions, aLMFs and HSC secreted high  
515 levels of IL-6, HGF, VEGF, CCL2, and CXCL8 under control conditions and  
516 stimulation with pro-inflammatory cytokines TNF $\alpha$  and IFN $\gamma$  enhanced all factors and  
517 induced secretion of additional chemokines including CCL5, CXCL9 and CXCL10.  
518 Moreover, aLMF- and HSC-conditioned supernatants promoted strong and rapid  
519 migration of lymphocytes towards these chemotactic factors under pro-inflammatory  
520 conditions and stimulated increased recruitment of lymphocytes across adjacent  
521 LSEC monolayers. These findings demonstrated that there are signals from HSCs  
522 which can recruit infiltrating immune cells which may be targeted to halt the  
523 progression of fibrogenesis. One such target which we have already discussed in the  
524 context of inflammation is VAP-1. VAP-1 is a dual functioning entity which, as

described, acts as an adhesion molecule as well as an enzyme which has a role in recruiting lymphocytes across endothelial cells<sup>80</sup>. More recent *in vivo* studies described a novel role of VAP-1 in hepatic inflammation and fibrogenesis through modulating HSC phenotype<sup>116</sup>. Soluble VAP-1 secreted by HSCs was enzymatically active and was able to recruit lymphocytes. VAP-1 modulation in the HSC cell line LX-2 increased transcription of profibrogenic genes such as collagen 1a1 as well as enhancing wound healing. These data were supported by murine models of liver injury in which VAP-1 knockout animals had less inflammation and fibrosis in response to injury<sup>116</sup>. The blockade of VAP-1 to treat primary sclerosing cholangitis (PSC) is currently being evaluated in the phase II clinical trial BUTEO (BUTEO NCT02239211).

## **Inflammatory pathways which promote fibrosis resolution and liver regeneration**

We have covered some of the mechanisms which drive effector immune responses within the liver but it is also becoming clear that pathways which promote resolution of the inflammatory process play a key role in determining the severity of tissue injury. Targeting cellular populations that promote resolution could provide a novel anti-inflammatory approach. The resolution of inflammation and fibrosis is a highly co-ordinated, multifaceted process that is intended to eliminate remaining injurious agents responsible for the initial insult and shift the balance from a pro-inflammatory to an anti-inflammatory microenvironment (Figure 3). This is achieved through a sequence of events where selected immune cell populations are removed through apoptosis/necrosis/efferocytosis accompanied by recruitment and differentiation of pro-resolution immune subsets such as macrophages. Homeostasis is then restored

following repopulation of the injured area through regeneration of the hepatocyte pool, repopulation of the Kupffer cell niche and maintenance of hepatic tolerance, for example through T<sub>reg</sub> recruitment and retention.

#### *Immune cell intervention*

Resolution of fibrosis is usually ascribed to the function of a specific macrophage population that secrete a range of pro-resolution mediators including matrix metalloproteinases, such as MMP-13<sup>117</sup>, which promote the degradation of scar tissue. Duffield and co-workers used a transgenic CD11b-DTR mouse to selectively deplete CD11b<sup>hi</sup> macrophages in a reversible CCl<sub>4</sub>-induced model of liver injury and described a biphasic injurious response; depletion of macrophages during ongoing injury reduced the extent of tissue damage, whereas depletion of the macrophage population following withdrawal of the toxin delayed recovery<sup>118</sup>. Building on these preliminary observations, hepatic macrophages have been shown to transition from pro-inflammatory Ly6C<sup>hi</sup>CCR2<sup>hi</sup>CX<sub>3</sub>CR1<sup>lo</sup> expressing populations to pro-reparative Ly6C<sup>lo</sup>CCR2<sup>lo</sup>CX<sub>3</sub>CR1<sup>hi</sup> subsets in mice, a process thought to be dependent on IL-4, IL-10 and phagocytosis<sup>24,119</sup>. Development of cellular therapy for liver cirrhosis through the provision of human phagocytic macrophage populations (CD163<sup>hi</sup>CD169<sup>hi</sup>CD206<sup>hi</sup>CCR2<sup>lo</sup>) is underway, with potential advantages over conventional monotherapeutic intervention strategies<sup>120,121</sup>.

Adhesion receptors may also play a dual role in both the establishment and resolution of hepatic injury. Stabilin-1 has been discussed in the context of leukocyte recruitment, but this molecule is also expressed by a highly phagocytic macrophage population during resolution of chronic liver disease where it serves to limit further



575 inflammation and fibrosis by scavenging products of lipid peroxidation and  
576 suppressing secretion of CCL3<sup>122</sup>. Similar roles for other scavenger receptors are  
577 highly likely within the context of inflammatory liver disease<sup>123</sup>.

578

579 Bile acids can signal through two major receptor pathways that regulate hepatic lipid  
580 and glucose metabolism, namely farnesoid X receptor (FXR) and TGR5 (a G protein-  
581 coupled bile acid receptor). Treatment of mice with the dual FXR/TGR5 agonist INT-  
582 767 induced a restorative intrahepatic macrophage phenotype (Ly6C<sup>lo</sup>CD206<sup>hi</sup> and  
583 expression of *Retnla* and *Clec7a*)<sup>124</sup>. Provision of agonists for FXR and TGR5 have  
584 been suggested as potential therapeutics during liver regeneration where there is an  
585 excess bile acid pool<sup>125</sup> in NASH<sup>126</sup> or in cholestatic liver diseases<sup>127</sup> although some  
586 caution is required given the pleiotropic effects of these receptors, such as the role of  
587 TGR5 in the development of cholangiocarcinoma<sup>128</sup>.

588

589 During acute liver failure (ALF), a marked increase in inflammatory macrophages is  
590 observed in areas of necrosis. However, patients with ALF exhibit an expanded  
591 population of macrophages with a resolution-like phenotype with suppressed innate  
592 and enhanced efferocytic/phagocytic responses that are present in both circulatory  
593 and tissue compartments. This functional switch was associated with the expression  
594 of the TAM family member Mer tyrosine kinase (MerTK<sup>+</sup>HLA-DR<sup>high</sup>) induced by  
595 secretory leukocyte protease inhibitor (SLPI) produced within the inflamed liver of  
596 both mice and humans following ALF. Such reprogramming of the myeloid  
597 population promotes neutrophil apoptosis and subsequent clearance through  
598 enhanced efferocytosis, and may be a target for future therapies<sup>129</sup>. Hepatocytes  
599 (and other liver resident cells) are also able to remove apoptotic and necrotic cells by

efferocytosis, although the relative contributions of this process to the resolution of chronic liver injury has not been determined fully<sup>130</sup>.

Macrophages are not the sole mediators of hepatic resolution. NK cell cytotoxicity against early-activated or senescent-activated HSC via NK cell activating ligands (RAE-1 in mice; MICA in human), TRAIL receptors and production of IFN- $\gamma$ , an inhibitor of HSC activation, promotes the resolution of liver injury<sup>131</sup>. Invariant NKT cells are thought to promote HSC killing, but can also be activated at the site of injury by self-antigens, leading to the production of IL-4 (but not IFN- $\gamma$ ), driving hepatocyte proliferation, a shift in the macrophage population from Ly6C<sup>hi</sup> to Ly6C<sup>lo</sup> expression and improved healing responses<sup>132</sup>. In mice, the regeneration of LSEC is dependent on the relative expression of the CXCL12 receptors CXCR4-7. During injury constitutive FGFR1 signalling increased the ratio of CXCR4: CXCR7 expression by LSEC, leading to an altered angiocrine response and proliferation of the stromal cell niche. Conversely, during resolution CXCR7 upregulation acts in concert with CXCR4 to induce the transcription factor Id1 with concomitant release of regenerative angiocrine factors and promotion of a pro-resolution environment<sup>133</sup>.

### *Hepatic regeneration*

Cellular repopulation of the hepatic niche following injury is essential to maintain not only the metabolic function of the organ, but also the ability to detoxify xenobiotics. Regeneration of the hepatocyte population is promoted by Kupffer cells through the production of IL-6 and TNF- $\alpha$ , driven by local recruitment of neutrophils in an ICAM-1 dependent process<sup>134-136</sup>, production of complement proteins C3a and C5a<sup>137</sup> and local provision of growth factors such as HGF, VEGF and IL-1a<sup>138</sup>. Repopulation of

the hepatic niche usually occurs through self-replication of hepatocytes; however, in chronic liver disease hepatocyte proliferation is often impaired (for example through immune cell-derived IFN- $\gamma$ <sup>131,139,140</sup>). Under these circumstances, the hepatocyte pool may be supplemented through a ductular reaction that regenerates functional hepatocytes from biliary cells, with important implications for therapeutic restoration of liver function<sup>141</sup>.

## Conclusion

We have highlighted several pathways and targets which could potentially contribute to new therapies for inflammatory liver disease. It is likely that combination therapies will be required to achieve significant clinical end points in terms of fibrosis regression and improvement in overall survival. An additional consideration is the dynamic and complex cycle of maldaptive wound repair which characterises advanced liver disease. It will be crucial that anti-inflammatory treatment for liver disease involves a personalised/precision medicine approach taking into account the stage of disease, inflammatory infiltrate and potential of driving fibrosis resolution. Whilst the benefits of inhibiting inflammation and driving resolution in chronic liver diseases are clear, the chronic nature of most liver diseases and the unique microenvironment of the liver promote the development of HCC. The future of developing novel anti-inflammatory agents in liver disease needs to take into account the potential of promoting HCC in the setting of subclinical malignancy or carcinoma-*in situ*. Previous studies have highlighted this potential risk in the setting of hepatitis C eradication with direct acting anti-viral therapy<sup>142</sup> and it is now becoming clear that HCC thrives in immunosuppressive microenvironments<sup>143</sup>. It is therefore important that we dedicate further research into understanding in which situations the

approach of suppressing inflammation in patients who have suffered liver disease for many years could potentially promote HCC. Nevertheless, we remain hopeful that the progress which has been made in understanding the regulators of inflammation in the liver microenvironment will lead to successful therapies to prevent the progression/reverse chronic liver disease.

## Figure Legends

### Figure 1 **Immune response to danger signals released from chronic epithelial injury**

Chronic epithelial damage in the liver leads to cellular stress and the release of danger signals. Pro-inflammatory pathways are triggered by Kupffer cell recognition of these danger signals by receptors including TLR-4, galectin 3 and CD36 as well as activation of the inflammasome. Subsequent recruitment of CCR2<sup>+</sup> monocytes into liver tissue from the circulation leads to exacerbation of fibrogenesis. Unconventional T cells also play an important role in sensing cellular stress at epithelial surfaces. CCR6<sup>+</sup>  $\gamma\delta$  T cells prevent fibrosis by promoting hepatic stellate cell apoptosis whereas NKT cells and MAIT promote fibrogenesis with NKT cells releasing pro fibrogenic factors such as osteopontin and hedgehog ligands and MAIT cells activating proinflammatory and profibrogenic pathways in macrophages and hepatic stellate cells. DAMPS, danger associated molecular patterns; HMGB1, high mobility group protein B1; MDA-LDL, Malondialdehyde- low density lipoprotein; ATP, adenosine triphosphate; NLRP3, NOD-, LRR- and pyrin domain-containing 3; NKT cell, natural killer T cell; MAIT cell, mucosal associated invariant T cell; HSC, hepatic stellate cell; ECM; extracellular matrix.

**Figure 2 Lymphocyte recruitment and retention within the hepatic sinusoids during chronic liver injury**

All progressive chronic inflammatory liver diseases are associated with recruitment and retention of circulating lymphocytes into liver tissue. This recruitment occurs within the low shear stress environment of the hepatic sinusoids, where lymphocyte recruitment is triggered by selectin-independent capture and firm adhesion by VCAM-1 supported by CD151 on the endothelial surface. Other factors promote lymphocyte subset specific recruitment including aberrant adhesion of gut-homing lymphocytes ( $\alpha 4\beta 7^+$ ) to MAdCAM-1 and CD4 lymphocytes adhesion mediated by SCARF1. Presentation of chemokines including IP-10 to CXCR3<sup>+</sup> T cells and CXCL16 to CXCR6<sup>+</sup> T cells triggers activation and migration of T cells. The subsequent transendothelial migration step involves a combination of receptors including the atypical adhesion molecule VAP-1 with Treg specific recruitment occurring via transcellular pathway mediated by VAP-1 and stabilin-1. HSCs contribute to subendothelial retention of lymphocytes through the release of several chemotactic factors and contribution from VAP-1. T cell subset positioning in liver tissue is further regulated by chemokines including CCL20 for Th17 cells and CCL17 and CCL22 for Tregs. VCAM-1, vascular adhesion molecule-1; MAdCAM-1, mucosal vascular addressin cell adhesion molecule-1; SCARF1, scavenger receptor class F, member 1; IP-10, interferon gamma-induced protein 10; VAP-1, vascular adhesion protein-1.

**Figure 3 Pathways which promote fibrosis resolution and liver regeneration**

The liver has the capacity to promote resolution of fibrosis and regenerative pathways. Kupffer cells have the capability to promote hepatocyte regeneration

through the release of several factors including IL-6 and TNF $\alpha$ . Liver sinusoidal endothelium can promote a pro regenerative pathway rather than pro-fibrotic through the upregulation of CXCR7 which induces the transcription factor Id1 leading to proregenerative angiocrine factors. NK cells can contribute to fibrosis resolution by directly killing senescence activated HSCs. Macrophages also play a pivotal role in fibrosis resolution through the release of several factors including MMP13 which degrades scar tissue. A key role is played by a subset of macrophages characterised by the pro-resolution phenotype Ly6C<sup>lo</sup>CCR2<sup>lo</sup>CX<sub>3</sub>CR1<sup>hi</sup>. In chronic liver injury, uptake of products of lipid peroxidation such as oxLDLs by macrophages expressing stabilin-1 suppresses the release of pro-fibrotic factors. During acute liver injury the release of SLPI leads to the upregulation of MerTK on macrophages which promotes neutrophil apoptosis and subsequent clearance leading to resolution of inflammation. NK cell, natural killer cell; MMP-13, metalloproteinase-13; oxLDL, oxidised low density lipoprotein; SLPI, secretory leukocyte protease inhibitor; MerTK, Mer tyrosine kinase.

1. Pimpin L, Cortez-Pinto H, Negro F, et al. Burden of liver disease in Europe: epidemiology and analysis of risk factors to identify prevention policies. *J Hepatol.* 2018.
2. Chung RT, Baumert TF. Curing chronic hepatitis C--the arc of a medical triumph. *N Engl J Med.* 2014;370(17):1576-1578.
3. Chang TT, Liaw YF, Wu SS, et al. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. *Hepatology.* 2010;52(3):886-893.
4. Papatheodoridis GV, Lampertico P, Manolakopoulos S, Lok A. Incidence of hepatocellular carcinoma in chronic hepatitis B patients receiving nucleos(t)ide therapy: a systematic review. *J Hepatol.* 2010;53(2):348-356.

- 730 5. Ramachandran P, Henderson NC. Antifibrotics in chronic liver disease: tractable  
731 targets and translational challenges. *Lancet Gastroenterol Hepatol*. 2016;1(4):328-  
732 340.
- 733 6. Canbay A, Friedman S, Gores GJ. Apoptosis: the nexus of liver injury and fibrosis.  
734 *Hepatology*. 2004;39(2):273-278.
- 735 7. Shetty S, Lalor PF, Adams DH. Lymphocyte recruitment to the liver: molecular  
736 insights into the pathogenesis of liver injury and hepatitis. *Toxicology*.  
737 2008;254(3):136-146.
- 738 8. Ibrahim SH, Hirsova P, Gores GJ. Non-alcoholic steatohepatitis pathogenesis:  
739 sublethal hepatocyte injury as a driver of liver inflammation. *Gut*. 2018;67(5):963-  
740 972.
- 741 9. Hirschfield GM, Karlsen TH, Lindor KD, Adams DH. Primary sclerosing cholangitis.  
742 *Lancet*. 2013;382(9904):1587-1599.
- 743 10. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. *Nat Rev*  
744 *Gastroenterol Hepatol*. 2017;14(7):397-411.
- 745 11. Lo RC, Kim H. Histopathological evaluation of liver fibrosis and cirrhosis regression.  
746 *Clin Mol Hepatol*. 2017;23(4):302-307.
- 747 12. Webb GJ, Siminovitch KA, Hirschfield GM. The immunogenetics of primary biliary  
748 cirrhosis: A comprehensive review. *J Autoimmun*. 2015;64:42-52.
- 749 13. Kubes P, Mehal WZ. Sterile inflammation in the liver. *Gastroenterology*.  
750 2012;143(5):1158-1172.
- 751 14. Krenkel O, Tacke F. Liver macrophages in tissue homeostasis and disease. *Nat Rev*  
752 *Immunol*. 2017;17(5):306-321.
- 753 15. Iacobini C, Menini S, Ricci C, et al. Accelerated lipid-induced atherogenesis in  
754 galectin-3-deficient mice: role of lipoxidation via receptor-mediated mechanisms.  
755 *Arterioscler Thromb Vasc Biol*. 2009;29(6):831-836.
- 756 16. Harrison SA, Marri SR, Chalasani N, et al. Randomised clinical study: GR-MD-02, a  
757 galectin-3 inhibitor, vs. placebo in patients having non-alcoholic steatohepatitis with  
758 advanced fibrosis. *Aliment Pharmacol Ther*. 2016;44(11-12):1183-1198.
- 759 17. Bieghs V, Wouters K, van Gorp PJ, et al. Role of scavenger receptor A and CD36 in  
760 diet-induced nonalcoholic steatohepatitis in hyperlipidemic mice. *Gastroenterology*.  
761 2010;138(7):2477-2486, 2486 e2471-2473.
- 762 18. Busch CJ, Hendriks T, Weismann D, et al. Malondialdehyde epitopes are sterile  
763 mediators of hepatic inflammation in hypercholesterolemic mice. *Hepatology*.  
764 2017;65(4):1181-1195.
- 765 19. Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease,  
766 and therapeutics. *Nat Med*. 2015;21(7):677-687.
- 767 20. Wree A, Marra F. The inflammasome in liver disease. *J Hepatol*. 2016;65(5):1055-  
768 1056.
- 769 21. Petrasek J, Bala S, Csak T, et al. IL-1 receptor antagonist ameliorates inflammasome-  
770 dependent alcoholic steatohepatitis in mice. *J Clin Invest*. 2012;122(10):3476-3489.
- 771 22. Henao-Mejia J, Elinav E, Jin C, et al. Inflammasome-mediated dysbiosis regulates  
772 progression of NAFLD and obesity. *Nature*. 2012;482(7384):179-185.
- 773 23. Wree A, McGeough MD, Inzaugarat ME, et al. NLRP3 inflammasome driven liver  
774 injury and fibrosis: Roles of IL-17 and TNF in mice. *Hepatology*. 2017.

- 775 24. Dal-Secco D, Wang J, Zeng Z, et al. A dynamic spectrum of monocytes arising from  
776 the in situ reprogramming of CCR2<sup>+</sup> monocytes at a site of sterile injury. *J Exp Med*.  
777 2015;212(4):447-456.
- 778 25. Karlmark KR, Weiskirchen R, Zimmermann HW, et al. Hepatic recruitment of the  
779 inflammatory Gr1<sup>+</sup> monocyte subset upon liver injury promotes hepatic fibrosis.  
780 *Hepatology*. 2009;50(1):261-274.
- 781 26. Tacke F. Targeting hepatic macrophages to treat liver diseases. *J Hepatol*.  
782 2017;66(6):1300-1312.
- 783 27. Krenkel O, Puengel T, Govaere O, et al. Therapeutic inhibition of inflammatory  
784 monocyte recruitment reduces steatohepatitis and liver fibrosis. *Hepatology*.  
785 2018;67(4):1270-1283.
- 786 28. Friedman SL, Ratziu V, Harrison SA, et al. A randomized, placebo-controlled trial of  
787 cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. *Hepatology*.  
788 2018;67(5):1754-1767.
- 789 29. Wang J, Kubes P. A Reservoir of Mature Cavity Macrophages that Can Rapidly Invade  
790 Visceral Organs to Affect Tissue Repair. *Cell*. 2016;165(3):668-678.
- 791 30. Kaufmann SH. gamma/delta and other unconventional T lymphocytes: what do they  
792 see and what do they do? *Proc Natl Acad Sci U S A*. 1996;93(6):2272-2279.
- 793 31. Kenna T, Golden-Mason L, Norris S, Hegarty JE, O'Farrelly C, Doherty DG. Distinct  
794 subpopulations of gamma delta T cells are present in normal and tumor-bearing  
795 human liver. *Clin Immunol*. 2004;113(1):56-63.
- 796 32. Carding SR, Egan PJ. Gammadelta T cells: functional plasticity and heterogeneity. *Nat*  
797 *Rev Immunol*. 2002;2(5):336-345.
- 798 33. Zhao N, Hao J, Ni Y, et al. Vgamma4 gammadelta T cell-derived IL-17A negatively  
799 regulates NKT cell function in Con A-induced fulminant hepatitis. *J Immunol*.  
800 2011;187(10):5007-5014.
- 801 34. Hammerich L, Bangen JM, Govaere O, et al. Chemokine receptor CCR6-dependent  
802 accumulation of gammadelta T cells in injured liver restricts hepatic inflammation  
803 and fibrosis. *Hepatology*. 2014;59(2):630-642.
- 804 35. Geissmann F, Cameron TO, Sidobre S, et al. Intravascular immune surveillance by  
805 CXCR6<sup>+</sup> NKT cells patrolling liver sinusoids. *PLoS Biol*. 2005;3(4):e113.
- 806 36. Wehr A, Baeck C, Heymann F, et al. Chemokine receptor CXCR6-dependent hepatic  
807 NK T Cell accumulation promotes inflammation and liver fibrosis. *J Immunol*.  
808 2013;190(10):5226-5236.
- 809 37. Syn WK, Oo YH, Pereira TA, et al. Accumulation of natural killer T cells in progressive  
810 nonalcoholic fatty liver disease. *Hepatology*. 2010;51(6):1998-2007.
- 811 38. Syn WK, Agboola KM, Swiderska M, et al. NKT-associated hedgehog and osteopontin  
812 drive fibrogenesis in non-alcoholic fatty liver disease. *Gut*. 2012;61(9):1323-1329.
- 813 39. Kurioka A, Walker LJ, Klenerman P, Willberg CB. MAIT cells: new guardians of the  
814 liver. *Clin Transl Immunology*. 2016;5(8):e98.
- 815 40. Kjer-Nielsen L, Patel O, Corbett AJ, et al. MR1 presents microbial vitamin B  
816 metabolites to MAIT cells. *Nature*. 2012;491(7426):717-723.
- 817 41. Treiner E, Duban L, Bahram S, et al. Selection of evolutionarily conserved mucosal-  
818 associated invariant T cells by MR1. *Nature*. 2003;422(6928):164-169.
- 819 42. Riva A, Patel V, Kurioka A, et al. Mucosa-associated invariant T cells link intestinal  
820 immunity with antibacterial immune defects in alcoholic liver disease. *Gut*.  
821 2018;67(5):918-930.



- 822 43. Tang XZ, Jo J, Tan AT, et al. IL-7 licenses activation of human liver intrasinusoidal  
823 mucosal-associated invariant T cells. *J Immunol.* 2013;190(7):3142-3152.
- 824 44. Jeffery HC, van Wilgenburg B, Kurioka A, et al. Biliary epithelium and liver B cells  
825 exposed to bacteria activate intrahepatic MAIT cells through MR1. *J Hepatol.*  
826 2016;64(5):1118-1127.
- 827 45. Balmer ML, Slack E, de Gottardi A, et al. The liver may act as a firewall mediating  
828 mutualism between the host and its gut commensal microbiota. *Sci Transl Med.*  
829 2014;6(237):237ra266.
- 830 46. Hegde P, Weiss E, Paradis V, et al. Mucosal-associated invariant T cells are a  
831 profibrogenic immune cell population in the liver. *Nat Commun.* 2018;9(1):2146.
- 832 47. Nourshargh S, Alon R. Leukocyte migration into inflamed tissues. *Immunity.*  
833 2014;41(5):694-707.
- 834 48. Braet F, Wisse E. Structural and functional aspects of liver sinusoidal endothelial cell  
835 fenestrae: a review. *Comp Hepatol.* 2002;1(1):1.
- 836 49. Sørensen KK, McCourt P, Berg T, et al. The scavenger endothelial cell: a new player in  
837 homeostasis and immunity. *American Journal of Physiology-Regulatory, Integrative*  
838 *and Comparative Physiology.* 2012;303(12):R1217-R1230.
- 839 50. Lalor P, Lai W, Curbishley S, Shetty S, Adams D. Human hepatic sinusoidal endothelial  
840 cells can be distinguished by expression of phenotypic markers related to their  
841 specialised functions in vivo. *World J Gastroenterol.* 2006;12(34):5429-5439.
- 842 51. McEver RP. Selectins: initiators of leucocyte adhesion and signalling at the vascular  
843 wall. *Cardiovascular research.* 2015;107(3):331-339.
- 844 52. Schnoor M, Alcaide P, Voisin MB, van Buul JD. Crossing the Vascular Wall: Common  
845 and Unique Mechanisms Exploited by Different Leukocyte Subsets during  
846 Extravasation. *Mediators Inflamm.* 2015;2015:946509.
- 847 53. Muller WA. Transendothelial migration: unifying principles from the endothelial  
848 perspective. *Immunol Rev.* 2016;273(1):61-75.
- 849 54. Shetty S, Weston CJ, Oo YH, et al. Common lymphatic endothelial and vascular  
850 endothelial receptor-1 mediates the transmigration of regulatory T cells across  
851 human hepatic sinusoidal endothelium. *J Immunol.* 2011;186(7):4147-4155.
- 852 55. Patten DA, Wilson GK, Bailey D, et al. Human liver sinusoidal endothelial cells  
853 promote intracellular crawling of lymphocytes during recruitment: A new step in  
854 migration. *Hepatology.* 2017;65(1):294-309.
- 855 56. Shetty S, Lalor PF, Adams DH. Lymphocyte recruitment to the liver: molecular  
856 insights into the pathogenesis of liver injury and hepatitis. *Toxicology.*  
857 2008;254(3):136-146.
- 858 57. Shetty S, Lalor PF, Adams DH. Liver sinusoidal endothelial cells—gatekeepers of  
859 hepatic immunity. *Nature Reviews Gastroenterology & Hepatology.* 2018;1.
- 860 58. Elices MJ, Osborn L, Takada Y, et al. VCAM-1 on activated endothelium interacts with  
861 the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding  
862 site. *Cell.* 1990;60(4):577-584.
- 863 59. Lalor PF, Clements JM, Pigott R, Humphries MJ, Spragg JH, Nash GB. Association  
864 between receptor density, cellular activation, and transformation of adhesive  
865 behavior of flowing lymphocytes binding to VCAM-1. *EurJImmunol.* 1997;27(6):1422-  
866 1426.
- 867 60. Lalor PF, Shields P, Grant A, Adams DH. Recruitment of lymphocytes to the human  
868 liver. *Immunol Cell Biol.* 2002;80(1):52-64.

- 869 61. Marlin SD, Springer TA. Purified intercellular adhesion molecule-1 (ICAM-1) is a  
870 ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell*. 1987;51(5):813-  
871 819.
- 872 62. Shetty S, Bruns T, Weston CJ, et al. Recruitment mechanisms of primary and  
873 malignant B cells to the human liver. *Hepatology*. 2012;56(4):1521-1531.
- 874 63. Haraldsen G, Kvale D, Lien B, Farstad IN, Brandtzaeg P. Cytokine-regulated  
875 expression of E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular  
876 cell adhesion molecule-1 (VCAM-1) in human microvascular endothelial cells. *The*  
877 *Journal of Immunology*. 1996;156(7):2558-2565.
- 878 64. Barreiro O, Zamai M, Yáñez-Mó M, et al. Endothelial adhesion receptors are  
879 recruited to adherent leukocytes by inclusion in preformed tetraspanin  
880 nanoplateforms. *The Journal of cell biology*. 2008;183(3):527-542.
- 881 65. Barreiro O, Yáñez-Mó M, Sala-Valdés M, et al. Endothelial tetraspanin microdomains  
882 regulate leukocyte firm adhesion during extravasation. *Blood*. 2005;105(7):2852-  
883 2861.
- 884 66. Wadkin JCR, Patten DA, Kamarajah S, et al. CD151 supports VCAM-1 mediated  
885 lymphocyte adhesion to liver endothelium and is upregulated in chronic liver disease  
886 and hepatocellular carcinoma. *Am J Physiol Gastrointest Liver Physiol*. 2017;ajpgi  
887 00411 02016.
- 888 67. Berlin C, Berg EL, Briskin MJ, et al.  $\alpha 4\beta 7$  integrin mediates lymphocyte binding to the  
889 mucosal vascular addressin MAdCAM-1. *Cell*. 1993;74(1):185-195.
- 890 68. Habtezion A, Nguyen LP, Hadeiba H, Butcher EC. Leukocyte Trafficking to the Small  
891 Intestine and Colon. *Gastroenterology*. 2016;150(2):340-354.
- 892 69. Liaskou E, Karikoski M, Reynolds GM, et al. Regulation of mucosal addressin cell  
893 adhesion molecule 1 expression in human and mice by vascular adhesion protein 1  
894 amine oxidase activity. *Hepatology*. 2011;53(2):661-672.
- 895 70. Grant AJ, Lalor PF, Hubscher SG, Briskin M, Adams DH. MAdCAM-1 expressed in  
896 chronic inflammatory liver disease supports mucosal lymphocyte adhesion to  
897 hepatic endothelium (MAdCAM-1 in chronic inflammatory liver disease).  
898 *Hepatology*. 2001;33(5):1065-1072.
- 899 71. Grant AJ, Lalor PF, Salmi M, Jalkanen S, Adams DH. Homing of mucosal lymphocytes  
900 to the liver in the pathogenesis of hepatic complications of inflammatory bowel  
901 disease. *Lancet*. 2002;359(9301):150-157.
- 902 72. Feagan BG, Rutgeerts P, Sands BE, et al. Vedolizumab as induction and maintenance  
903 therapy for ulcerative colitis. *N Engl J Med*. 2013;369(8):699-710.
- 904 73. Sandborn WJ, Feagan BG, Rutgeerts P, et al. Vedolizumab as induction and  
905 maintenance therapy for Crohn's disease. *N Engl J Med*. 2013;369(8):711-721.
- 906 74. Lim TY, Pavlidis P, Gulati S, et al. Vedolizumab in Inflammatory Bowel Disease  
907 Associated with Autoimmune Liver Disease Pre- and Postliver Transplantation: A  
908 Case Series. *Inflamm Bowel Dis*. 2016;22(10):E39-40.
- 909 75. Christensen B, Micic D, Gibson PR, et al. Vedolizumab in patients with concurrent  
910 primary sclerosing cholangitis and inflammatory bowel disease does not improve  
911 liver biochemistry but is safe and effective for the bowel disease. *Aliment Pharmacol*  
912 *Ther*. 2018;47(6):753-762.
- 913 76. Salmi M, Jalkanen S. Vascular Adhesion Protein-1: A Cell Surface Amine Oxidase in  
914 Translation. *Antioxid Redox Signal*. 2017.

- 915 77. Jaakkola K, Nikula T, Holopainen R, et al. In vivo detection of vascular adhesion  
916 protein-1 in experimental inflammation. *The American journal of pathology*.  
917 2000;157(2):463-471.
- 918 78. Salmi M, Tohka S, Berg EL, Butcher EC, Jalkanen S. Vascular adhesion protein 1 (VAP-  
919 1) mediates lymphocyte subtype-specific, selectin-independent recognition of  
920 vascular endothelium in human lymph nodes. *JExpMed*. 1997;186(4):589-600.
- 921 79. McNab G, Reeves J, Salmi M, Hubscher S, Jalkanen S, Adams D. Vascular adhesion  
922 protein 1 mediates binding of T cells to human hepatic endothelium.  
923 *Gastroenterology*. 1996;110(2):522-528.
- 924 80. Lalor PF, Edwards S, McNab G, Salmi M, Jalkanen S, Adams DH. Vascular adhesion  
925 protein-1 mediates adhesion and transmigration of lymphocytes on human hepatic  
926 endothelial cells. *J Immunol*. 2002;169(2):983-992.
- 927 81. Lalor PF, Sun PJ, Weston CJ, Martin-Santos A, Wakelam MJ, Adams DH. Activation of  
928 vascular adhesion protein-1 on liver endothelium results in an NF- $\kappa$ B-dependent  
929 increase in lymphocyte adhesion. *Hepatology*. 2007;45(2):465-474.
- 930 82. Weston CJ, Shepherd EL, Claridge LC, et al. Vascular adhesion protein-1 promotes  
931 liver inflammation and drives hepatic fibrosis. *The Journal of clinical investigation*.  
932 2015;125(2):501-520.
- 933 83. Salmi M, Jalkanen S. Vascular Adhesion Protein-1: A Cell Surface Amine Oxidase in  
934 Translation. *Antioxidants & redox signaling*. 2017.
- 935 84. Geissmann F, Cameron TO, Sidobre S, et al. Intravascular immune surveillance by  
936 CXCR6+ NKT cells patrolling liver sinusoids. *PLoS biology*. 2005;3(4):e113.
- 937 85. Wang H, Shao Y, Zhang S, et al. CXCL16 deficiency attenuates acetaminophen-  
938 induced hepatotoxicity through decreasing hepatic oxidative stress and  
939 inflammation in mice. *Acta biochimica et biophysica Sinica*. 2017;49(6):541-549.
- 940 86. Xu H, Xu W, Chu Y, Gong Y, Jiang Z, Xiong S. Involvement of up-regulated CXC  
941 chemokine ligand 16/scavenger receptor that binds phosphatidylserine and oxidized  
942 lipoprotein in endotoxin-induced lethal liver injury via regulation of T-cell  
943 recruitment and adhesion. *Infection and immunity*. 2005;73(7):4007-4016.
- 944 87. Heydtmann M, Lalor PF, Eksteen JA, Hübscher SG, Briskin M, Adams DH. CXC  
945 chemokine ligand 16 promotes integrin-mediated adhesion of liver-infiltrating  
946 lymphocytes to cholangiocytes and hepatocytes within the inflamed human liver.  
947 *The Journal of Immunology*. 2005;174(2):1055-1062.
- 948 88. Sato T, Thorlacius H, Johnston B, et al. Role for CXCR6 in recruitment of activated  
949 CD8+ lymphocytes to inflamed liver. *The Journal of Immunology*. 2005;174(1):277-  
950 283.
- 951 89. Hudspeth K, Donadon M, Cimino M, et al. Human liver-resident CD56bright/CD16neg  
952 NK cells are retained within hepatic sinusoids via the engagement of CCR5 and  
953 CXCR6 pathways. *Journal of autoimmunity*. 2016;66:40-50.
- 954 90. Stegmann KA, Robertson F, Hansi N, et al. CXCR6 marks a novel subset of T-bet lo  
955 Eomes hi natural killer cells residing in human liver. *Scientific reports*. 2016;6:26157.
- 956 91. Xu H-B, Gong Y-P, Cheng J, Chu Y-W, Xiong S-D. CXCL16 participates in pathogenesis  
957 of immunological liver injury by regulating T lymphocyte infiltration in liver tissue.  
958 *World journal of gastroenterology: WJG*. 2005;11(32):4979.
- 959 92. Wehr A, Tacke F. The Roles of CXCL16 and CXCR6 in Liver Inflammation and Fibrosis.  
960 *Current Pathobiology Reports*. 2015;3(4):283-290.

961 93. Irjala H, Elima K, Johansson EL, et al. The same endothelial receptor controls  
962 lymphocyte traffic both in vascular and lymphatic vessels. *European journal of*  
963 *immunology*. 2003;33(3):815-824.

964 94. Zhao H, Liao X, Kang Y. Tregs: Where We Are and What Comes Next? *Front Immunol*.  
965 2017;8:1578.

966 95. Politz O, Gratchev A, McCOURT PA, et al. Stabilin-1 and- 2 constitute a novel family  
967 of fasciclin-like hyaluronan receptor homologues. *Biochemical Journal*.  
968 2002;362(1):155-164.

969 96. Zhou B, Weigel JA, Fauss L, Weigel PH. Identification of the hyaluronan receptor for  
970 endocytosis (HARE). *Journal of Biological Chemistry*. 2000;275(48):37733-37741.

971 97. Jung M-Y, Park S-Y, Kim I-S. Stabilin-2 is involved in lymphocyte adhesion to the  
972 hepatic sinusoidal endothelium via the interaction with  $\alpha M\beta 2$  integrin. *Journal of*  
973 *leukocyte biology*. 2007;82(5):1156-1165.

974 98. Piccolo P, Vetrini F, Mithbaokar P, et al. SR-A and SREC-I are Kupffer and endothelial  
975 cell receptors for helper-dependent adenoviral vectors. *Mol Ther*. 2013;21(4):767-  
976 774.

977 99. Patten DA, Kamarajah SK, Rose JM, et al. SCARF-1 promotes adhesion of CD4(+) T  
978 cells to human hepatic sinusoidal endothelium under conditions of shear stress. *Sci*  
979 *Rep*. 2017;7(1):17600.

980 100. Patten DA. SCARF1: a multifaceted, yet largely understudied, scavenger receptor.  
981 *Inflammation Research*. 2018:1-6.

982 101. Campbell JJ, Hedrick J, Zlotnik A, Siani MA, Thompson DA, Butcher EC. Chemokines  
983 and the arrest of lymphocytes rolling under flow conditions. *Science*.  
984 1998;279(5349):381-384.

985 102. Shulman Z, Cohen SJ, Roediger B, et al. Transendothelial migration of lymphocytes  
986 mediated by intraendothelial vesicle stores rather than by extracellular chemokine  
987 depots. *Nat Immunol*. 2012;13(1):67-76.

988 103. Curbishley SM, Eksteen B, Gladue RP, Lalor P, Adams DH. CXCR 3 activation promotes  
989 lymphocyte transendothelial migration across human hepatic endothelium under  
990 fluid flow. *Am J Pathol*. 2005;167(3):887-899.

991 104. Nishioji K, Okanou T, Itoh Y, et al. Increase of chemokine interferon-inducible  
992 protein-10 (IP-10) in the serum of patients with autoimmune liver diseases and  
993 increase of its mRNA expression in hepatocytes. *Clin Exp Immunol*. 2001;123(2):271-  
994 279.

995 105. Butera D, Marukian S, Iwamaye AE, et al. Plasma chemokine levels correlate with the  
996 outcome of antiviral therapy in patients with hepatitis C. *Blood*. 2005;106(4):1175-  
997 1182.

998 106. Ajuebor MN, Hogaboam CM, Le T, Proudfoot AE, Swain MG. CCL3/MIP-1 $\alpha$  is pro-  
999 inflammatory in murine T cell-mediated hepatitis by recruiting CCR1-expressing  
1000 CD4(+) T cells to the liver. *Eur J Immunol*. 2004;34(10):2907-2918.

1001 107. Shields PL, Morland CM, Salmon M, Qin S, Hubscher SG, Adams DH. Chemokine and  
1002 chemokine receptor interactions provide a mechanism for selective T cell  
1003 recruitment to specific liver compartments within hepatitis C-infected liver. *J*  
1004 *Immunol*. 1999;163(11):6236-6243.

1005 108. de Graaf KL, Lapeyre G, Guilhot F, et al. NI-0801, an anti-chemokine (C-X-C motif)  
1006 ligand 10 antibody, in patients with primary biliary cholangitis and an incomplete  
1007 response to ursodeoxycholic acid. *Hepatol Commun*. 2018;2(5):492-503.

1008 109. Oo YH, Banz V, Kavanagh D, et al. CXCR3-dependent recruitment and CCR6-mediated  
1009 positioning of Th-17 cells in the inflamed liver. *J Hepatol.* 2012;57(5):1044-1051.  
1010 110. Oo YH, Weston CJ, Lalor PF, et al. Distinct roles for CCR4 and CXCR3 in the  
1011 recruitment and positioning of regulatory T cells in the inflamed human liver. *J*  
1012 *Immunol.* 2010;184(6):2886-2898.  
1013 111. Meng F, Wang K, Aoyama T, et al. Interleukin-17 signaling in inflammatory, Kupffer  
1014 cells, and hepatic stellate cells exacerbates liver fibrosis in mice. *Gastroenterology.*  
1015 2012;143(3):765-776 e763.  
1016 112. Rios DA, Valva P, Casciato PC, et al. Chronic hepatitis C liver microenvironment: role  
1017 of the Th17/Treg interplay related to fibrogenesis. *Sci Rep.* 2017;7(1):13283.  
1018 113. He B, Wu L, Xie W, et al. The imbalance of Th17/Treg cells is involved in the  
1019 progression of nonalcoholic fatty liver disease in mice. *BMC Immunol.* 2017;18(1):33.  
1020 114. Higashi T, Friedman SL, Hoshida Y. Hepatic stellate cells as key target in liver fibrosis.  
1021 *Adv Drug Deliv Rev.* 2017;121:27-42.  
1022 115. Holt AP, Haughton EL, Lalor PF, Filer A, Buckley CD, Adams DH. Liver myofibroblasts  
1023 regulate infiltration and positioning of lymphocytes in human liver.  
1024 *Gastroenterology.* 2009;136(2):705-714.  
1025 116. Weston CJ, Shepherd EL, Claridge LC, et al. Vascular adhesion protein-1 promotes  
1026 liver inflammation and drives hepatic fibrosis. *J Clin Invest.* 2015;125(2):501-520.  
1027 117. Fallowfield JA, Mizuno M, Kendall TJ, et al. Scar-associated macrophages are a major  
1028 source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine  
1029 hepatic fibrosis. *J Immunol.* 2007;178(8):5288-5295.  
1030 118. Duffield JS, Forbes SJ, Constandinou CM, et al. Selective depletion of macrophages  
1031 reveals distinct, opposing roles during liver injury and repair. *J Clin Invest.*  
1032 2005;115(1):56-65.  
1033 119. Ramachandran P, Pellicoro A, Vernon MA, et al. Differential Ly-6C expression  
1034 identifies the recruited macrophage phenotype, which orchestrates the regression of  
1035 murine liver fibrosis. *Proc Natl Acad Sci U S A.* 2012;109(46):E3186-3195.  
1036 120. Fraser AR, Pass C, Burgoyne P, et al. Development, functional characterization and  
1037 validation of methodology for GMP-compliant manufacture of phagocytic  
1038 macrophages: A novel cellular therapeutic for liver cirrhosis. *Cytotherapy.*  
1039 2017;19(9):1113-1124.  
1040 121. Moore JK, Mackinnon AC, Wojtacha D, et al. Phenotypic and functional  
1041 characterization of macrophages with therapeutic potential generated from human  
1042 cirrhotic monocytes in a cohort study. *Cytotherapy.* 2015;17(11):1604-1616.  
1043 122. Rantakari P, Patten DA, Valtonen J, et al. Stabilin-1 expression defines a subset of  
1044 macrophages that mediate tissue homeostasis and prevent fibrosis in chronic liver  
1045 injury. *Proc Natl Acad Sci U S A.* 2016;113(33):9298-9303.  
1046 123. Patten DA, Shetty S. Chronic liver disease: scavenger hunt for novel therapies.  
1047 *Lancet.* 2018;391(10116):104-105.  
1048 124. McMahan RH, Wang XX, Cheng LL, et al. Bile acid receptor activation modulates  
1049 hepatic monocyte activity and improves nonalcoholic fatty liver disease. *J Biol Chem.*  
1050 2013;288(17):11761-11770.  
1051 125. Merlen G, Ursic-Bedoya J, Jourdainne V, et al. Bile acids and their receptors during  
1052 liver regeneration: "Dangerous protectors". *Mol Aspects Med.* 2017;56:25-33.

1053 126. Roth JD, Feigh M, Veidal SS, et al. INT-767 improves histopathological features in a  
1054 diet-induced ob/ob mouse model of biopsy-confirmed non-alcoholic steatohepatitis.  
1055 *World J Gastroenterol.* 2018;24(2):195-210.

1056 127. Baghdasaryan A, Claudel T, Gumhold J, et al. Dual farnesoid X receptor/TGR5 agonist  
1057 INT-767 reduces liver injury in the Mdr2-/- (Abcb4-/-) mouse cholangiopathy model  
1058 by promoting biliary HCO<sup>-</sup>(3) output. *Hepatology.* 2011;54(4):1303-1312.

1059 128. Reich M, Deutschmann K, Sommerfeld A, et al. TGR5 is essential for bile acid-  
1060 dependent cholangiocyte proliferation in vivo and in vitro. *Gut.* 2016;65(3):487-501.

1061 129. Triantafyllou E, Pop OT, Possamai LA, et al. MerTK expressing hepatic macrophages  
1062 promote the resolution of inflammation in acute liver failure. *Gut.* 2018;67(2):333-  
1063 347.

1064 130. Davies SP, Reynolds GM, Stamataki Z. Clearance of Apoptotic Cells by Tissue  
1065 Epithelia: A Putative Role for Hepatocytes in Liver Efferocytosis. *Front Immunol.*  
1066 2018;9:44.

1067 131. Gao B, Radaeva S, Park O. Liver natural killer and natural killer T cells:  
1068 immunobiology and emerging roles in liver diseases. *J Leukoc Biol.* 2009;86(3):513-  
1069 528.

1070 132. Liew PX, Lee WY, Kubes P. iNKT Cells Orchestrate a Switch from Inflammation to  
1071 Resolution of Sterile Liver Injury. *Immunity.* 2017;47(4):752-765 e755.

1072 133. Ding BS, Cao Z, Lis R, et al. Divergent angiocrine signals from vascular niche balance  
1073 liver regeneration and fibrosis. *Nature.* 2014;505(7481):97-102.

1074 134. Selzner N, Selzner M, Odermatt B, Tian Y, Van Rooijen N, Clavien PA. ICAM-1 triggers  
1075 liver regeneration through leukocyte recruitment and Kupffer cell-dependent release  
1076 of TNF-alpha/IL-6 in mice. *Gastroenterology.* 2003;124(3):692-700.

1077 135. Cressman DE, Greenbaum LE, DeAngelis RA, et al. Liver failure and defective  
1078 hepatocyte regeneration in interleukin-6-deficient mice. *Science.*  
1079 1996;274(5291):1379-1383.

1080 136. Akerman P, Cote P, Yang SQ, et al. Antibodies to tumor necrosis factor-alpha inhibit  
1081 liver regeneration after partial hepatectomy. *Am J Physiol.* 1992;263(4 Pt 1):G579-  
1082 585.

1083 137. Strey CW, Markiewski M, Mastellos D, et al. The proinflammatory mediators C3a and  
1084 C5a are essential for liver regeneration. *J Exp Med.* 2003;198(6):913-923.

1085 138. Michalopoulos GK. Liver regeneration. *J Cell Physiol.* 2007;213(2):286-300.

1086 139. Sun R, Gao B. Negative regulation of liver regeneration by innate immunity (natural  
1087 killer cells/interferon-gamma). *Gastroenterology.* 2004;127(5):1525-1539.

1088 140. Shen K, Zheng SS, Park O, Wang H, Sun Z, Gao B. Activation of innate immunity  
1089 (NK/IFN-gamma) in rat allogeneic liver transplantation: contribution to liver injury  
1090 and suppression of hepatocyte proliferation. *Am J Physiol Gastrointest Liver Physiol.*  
1091 2008;294(4):G1070-1077.

1092 141. Raven A, Lu WY, Man TY, et al. Cholangiocytes act as facultative liver stem cells  
1093 during impaired hepatocyte regeneration. *Nature.* 2017;547(7663):350-354.

1094 142. Reig M, Marino Z, Perello C, et al. Unexpected high rate of early tumor recurrence in  
1095 patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol.*  
1096 2016;65(4):719-726.

1097 143. Hernandez-Gea V, Toffanin S, Friedman SL, Llovet JM. Role of the microenvironment  
1098 in the pathogenesis and treatment of hepatocellular carcinoma. *Gastroenterology.*  
1099 2013;144(3):512-527.

