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The immunophenotype of antigen presenting cells of the mononuclear phagocyte system in normal human liver – A systematic review

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Summary

The mononuclear phagocytic system (MPS), comprised of monocytes, macrophages, and dendritic cells, is essential in tissue homeostasis and in determining the balance of the immune response through its role in antigen presentation. It has been identified as a therapeutic target in infectious disease, cancer, autoimmune disease and transplant rejection. Here, we review the current understanding of the immunophenotype and function of the MPS in normal human liver. Using well-defined selection criteria, a search of MEDLINE and EMBASE databases identified 76 appropriate studies. The majority (n = 67) described Kupffer cells (KCs), although the definition of KC differs between sources, and little data were available regarding their function. Only 10 papers looked at liver dendritic cells (DCs), and largely confirmed the presence of the major dendritic cell subsets identified in human blood. Monocytes were thoroughly characterized in four studies that utilized flow cytometry and fluorescent microscopy and highlighted their prominent role in liver homeostasis and displayed subtle differences from circulating monocytes. There was some limited evidence that liver DCs are tolerogenic but neither liver dendritic cell subsets nor macrophages have been thoroughly characterized, using either multi-colour flow cytometry or multi-parameter fluorescence microscopy. The lobular distribution of different subsets of liver MPS cells was also poorly described, and the ability to distinguish between passenger leukocytes and tissue resident cells remains limited. It was apparent that further research, using modern immunological techniques, is now required to accurately characterize the cells of the MPS in human liver.

Introduction

The immune system is precisely balanced between immune activation and tolerance. Within this system antigen presenting cells (APCs) play a critical role in orchestrating the immune response [1].

In the normal liver the immunological balance is weighted towards a resting state of tolerance [2]. This immunological tolerance is seen in transplantation with a reduced rate of transplant rejection, even across MHC-disparate barriers [3]. It is also evident in infectious disease or cancer, with the liver readily harbouring chronic diseases such as hepatitis C, and both primary and secondary malignancies [4]. Despite this functional tolerant state, the liver can still under certain circumstances, such as transplant rejection, induce a potent immune response [5].

Animal studies show heterogeneous populations of liver APCs with varying functions that help to explain the liver's tolerogenic state and have identified these cells as potential therapeutic targets. A thorough understanding of the APCs in human liver will be required to enable their therapeutic manipulation.

Although a number of cell types within the liver have the potential to present antigens to T cells (broadly reviewed for animal and human liver in [6] and [7]) – including stellate cells (reviewed in [8]), endothelial cells (reviewed in [9]), and hepatocytes [7,10] – the intrahepatic cells of the mononuclear phagocyte system (MPS) play a major role in determining the nature of the immune response [6,11]. This review therefore focused on APCs within the MPS of the human liver, Table 1.

The MPS is composed of three major cells types – monocytes, macrophages, and dendritic cells (DCs) – although as a result of phenotypic and functional overlaps the precise boundaries, defining these groups, are not certain. A current theory regarding the

Table 1. Liver APC subsets, function and areas requiring further research.

Monocytes	**Molecularly defined** • CD14++CD16- • CD14++CD16+ • DC-like phenotype - High DR, CD80+ • Macrophage-like phenotype - CD163+, CD68+ • CD16+CD14dim [21-23] • CD14 "DC"-Postulated to be monocyte derived [98]	CD16+ monocytes (undefined as to whether they CD14++CD16+ or CD16+CD14dim) possess superior phagocytosis compared to blood monocytes and can efficiently activate CD4+ T cells [21-23]	Sinusoidal, throughout lobule [21-23]	• Functional assays of different CD14++CD16+ subsets • Accurate distinction between monocyte, DC, and LM • Identification of therapeutic targets
Liver macrophages	• Pan CD68 expression **Moleculary defined** Heterogeneity present but no clear subset definition using molecular markers except the presence of macrophage-like monocytes (see above) **Morphologically defined** • "Thin"-extended cytoplasm [32] • "Round"-larger cells with round cytoplasm [32] • 5 subsets defined by varying endogenous peroxidase activity and levels of endoplasmic reticulum [86]	• Minimal functional experimental evidence on liver macrophages • Liver Macrophages appear to be predominantly tolerogenic in nature, with a regulatory and scavenging role [41,57,69,89] • Function inferred through observations of variable expression of antigen presenting molecules [72], lectins [33,49,55,69,92], Fc Receptors, complement receptors [70], low co-stimulatory marker expression [77] and inhibitory markers such as Z39Ig [89]	Perisinusoidal [78]	• Further detail around subset definition and function • A variety of markers have been identified, but how this affects function is unknown, and how this changes according to subset is unknown • Identification of therapeutic targets
Dendritic cells	**Molecular defined** • BDCA1 (CD1c+) DC • BDCA2 (CD303+) DC • BDCA3 (CD141hi) DC Also two subsets defined as having high lipid and low lipid content [101]	**Tolerogenic in nature** • Lower expression of co-stimulation markers compared to spleen [98] • Produce IL-10 on LPS stimulation [98] • Stimulate T-cells that are IL-10 producing and hypo-responsive on re-stimulation [98] • Produce higher numbers of FoxP3+ Treg cells on naïve T cell stimulation [98] • Weak MLR response compared to blood [98]	Portal tract [103]	• Further subset distinction using transcription factors and markers expression • Functional assays of all subsets, including CD8+ T cell stimulation, response to gut-flora • Identification of therapeutic targets

Liver APCs can be divided into monocytes, macrophages, and dendritic cells. Monocytes are the most thoroughly investigated subset regarding accurate functional analysis and have a different proportional composition of monocyte subsets compared to blood. Macrophage heterogeneity remains undetermined and very little functional data exists for macrophages. Dendritic cells appear to represent the subsets found in blood but little functional data is available regarding these subsets.

ontogeny of the MPS, based on animal evidence, is summarized in Fig. 1.

Compared to other cells in the body, those of the MPS appear to be superior at sampling their environment through phagocytosis, and presenting antigen to T cells, especially to CD4+ T cells via MHC class II molecules (HLA-DP, -DQ, and -DR) [12]. APCs of the MPS appear to have a commensurately increased expression of antigen presentation and co-stimulatory molecules, and are potent secretors of modulatory cytokines [12]. They are motile,

and in other tissues have been shown to express chemokine receptors which facilitate their transit to draining lymph nodes, prime naïve T cells and establish a systemic immune response [13–15]. It is for these reasons that cells of MPS have become attractive targets to manipulate for therapy, as well as monitor for changes in disease states [16].

Interpreting experimental data regarding MPS cells in the liver requires an appreciation of their diversity. In blood and other tissues, monocytes, macrophages and DCs can be further

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divided into subsets with different potential for antigen presentation, motility, and cytokine production, all of which can help determine the nature of the immune system's response [17]. A particular complicating factor in the case of the liver is that circulating monocytes also traffic through the sinusoids, and distinguishing between *in transit* monocyte subsets and other MPS subsets is an evolving science [17]. Indeed the body's entire circulation of blood passes through the liver numerous times a day [18], so it is crucial to try and distinguish the role of MPS cells that are resident in the liver from those that are passing through it. In this review we were therefore careful to distinguish between findings that may relate to monocyte subsets *in transit* within the sinusoids rather than liver-resident MPS cells, especially those that are perisinusoidal, or reside in the portal tract.

Key Points 1

- Cells of the mononuclear phagocytic system (MPS) play a prominent role in antigen presentation to cells of the immune system locally and in draining lymph nodes

- Like other tissue, the human liver possesses all three major cells types of the MPS – monocytes, macrophages, and dendritic cells (DC). Liver monocytes and DC have a similar composition to blood but there is some evidence that they are more tolerogenic

- Liver macrophages have been broadly described as Kupffer cells, though they are clearly comprised of diverse subsets. Animal evidence indicates subsets of liver macrophages have distinct ontogeny and functions, but this concept has yet to be fully explored in humans

- New research is required to accurately define the subsets of MPS cells within normal human liver tissue and to adequately determine their function and their roles in liver homeostasis and pathology

- Better understanding of the liver MPS will enable the discovery of potential targets for immunotherapeutic intervention in liver disease and transplantation

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Methods

An electronic search was performed of the Medline and EMBASE databases from 1950 to July 2013 and 1980 to July 2013, respectively. Subject headings (MeSH) and truncated word searches were used for the following terms: [antigen present$, kupffer cell, macrophage, monocyte or dendritic cell] and [liver$ or hepat$]. Terms to incorporate the immunophenotype were based on methodology and included [histology, phenotype, immunophenotype, immunohistochemistry, flow cytometry or electron microscopy]. Studies were excluded if (i) the liver was diseased or transplanted, (ii) did not describe use of a normal human liver, or (iii) were not original research (systematic review, narrative review, commentary or editorial), Fig. 2. Articles were identified electronically using the above search strategy and eligible abstracts were screened manually by the primary reviewer (O. Strauss). Selected articles were retrieved and screened in depth for eligibility, and reference lists were manually checked for other potentially papers. Human studies focussing on diseased liver but also describing positive immunophenotypic findings of normal controls in liver tissue were included in the analysis. Duplicate studies were excluded and only articles published in the English language were included.

Results

Liver monocytes

Subsets and phenotype

Sinusoids of the liver contain circulating cells including monocytes. While some of these cells transit through the liver and return to the systemic circulation, others may adhere to the sinusoidal endothelium and ultimately differentiate into KCs [19].

Four papers described the immunophenotype of liver monocytes [20–23]. All the subsets of monocytes found in the blood are found in the liver.

Three major subclasses of monocytes are currently reported to exist in the blood [24]. The "classical" CD14$^+$CD16$^-$ subset, the "non-classical" CD14^{lo}CD16$^+$ subset, and the "intermediate" (CD14^{hi}CD16$^+$) [17] subset that appears to be in a transitional state between classical and non-classical monocytes [24]. *In vivo* monocytes can probably also differentiate into "CD14$^+$ DC" [25] that are HLA-DR^{hi}CD11c$^+$ but lack other DC markers, with a transcriptional profile closest to *in vitro* cultured monocyte-derived dendritic cells (MoDC). It is postulated that they are the *in vivo* equivalent of a MoDC and in keeping with this they possess many phenotypic and genomic characteristics of monocyte derived macrophages [26].

In the liver compared to blood, there is a decreased proportion of classical CD14^{hi}CD16$^-$ monocytes (80% *vs.* 50%), and an increased level of intermediate CD14^{hi}CD16$^+$ monocytes (9% *vs.* 27%), whereas the frequency of non-classical monocytes is unchanged [22]. The increased proportion of intermediate monocytes in the liver is thought to be due in part to the increased ability of CD16$^+$ monocytes to transmigrate across the hepatic endothelium as well as through an increase in local differentiation from classical CD14^{hi}CD16$^-$ monocytes as a result of the high levels of IL-10 and transforming growth factor beta. These intermediate (CD14^{hi}CD16$^+$) monocytes in the liver exhibit features of potent T cell stimulators, such as high HLA-DR, CD80, CD83, and CD86 [22]. They also express CD163, often described as a macrophage cell-surface marker, though it is also rapidly upregulated on the surface of activated monocytes.

As noted below, immunofluorescence microscopy also confirmed that some CD14$^+$, CD16$^+$, and CD14$^+$CD16$^+$ cells co-expressed CD68 – most commonly associated with macrophages. While the CD68$^-$ cells were probably monocytes *in transit* through the sinusoids, the expression of CD68$^+$ may indicate monocytes differentiating into tissue macrophages within the liver. Given that CD14 is also expressed by sinus-resident cells of the MPS in human lymph nodes [27], it is also possible that CD14 is expressed by at least some MPS cells that are resident in the liver sinusoids, as well as monocytes *in transit*. The current literature is unable to distinguish between these possibilities.

The most thorough investigation of liver monocytes reported only performed fluorescence-activated cell sorting (FACS) on CD16$^+$ monocytes (comprising both intermediate and non-classical monocytes) and showed that these cells could efficiently present antigen to autologous T cells [22]. This is in keeping with the finding that intermediate monocytes expressed high HLA-DR, CD80, CD83, and CD86. All three subsets of monocytes in the normal liver express CCR2 [21–23], and this is supported by liver tissue gene expression analysis [23]. As has been shown in studies in mice [19], this suggests that CCL2 (monocyte chemoattractant protein 1) will be a major mediator of monocyte recruitment
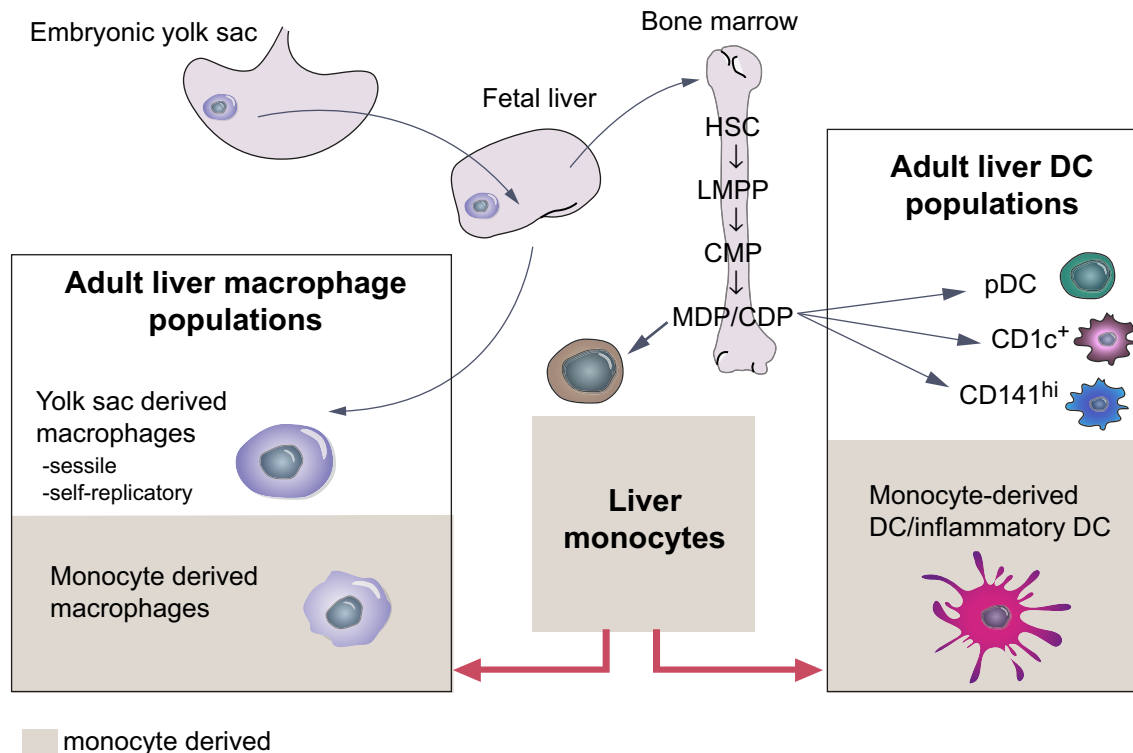
Fig. 1. Postulated ontogeny of liver antigen presenting subsets. Liver macrophages can broadly be defined as monocyte derived macrophages or self-replicating yolk-sac derived macrophages. The point at which a monocyte becomes a macrophage is not clearly defined. Liver dendritic cells (DCs) also contain a population of monocyte derived cells, these are important in inflammatory states. Liver DCs are also derived from immature blood dendritic cells that develop from a dendritic cell precursor. HSC, haematopoietic stem cell; LMPP, lymphoid-primed multipotent progenitor; CMP, common myeloid progenitor; MDP, macrophage and DC progenitor; CDP, common dendritic cell precursor; pDC, plasmacytoid dendritic cell.

into the liver in humans. Further to that, an *in vitro* model assessed the recruitment of human peripheral blood CD16^{+} monocytes across human liver sinusoidal endothelium and highlighted the expression and importance of CX_3CR1 in the transmigration of this subset of monocytes [20], however this has yet to be assessed on intrahepatic monocytes.

Kupffer cells (KCs)

Macrophages in the liver are generally described as Kupffer cells [28]. The small amount of human data and growing amount of mouse data, supporting heterogeneity of tissue macrophages in general [29] and KCs in particular [30], highlight the historical confusion about the accurate definition of these cells. In 1876 Karl von Kupffer described what he thought was the "phagosome rich" cell of the reticuloendothelial system; in fact he described liver stellate cells, with their large number of vitamin A-containing globules. It was only in 1970, that Wisse *et al.* used electron microscopy to clearly define the presence of peri-sinusoidal macrophage cells. Despite the misnomer, the term "Kupffer cell", has become synonymous with that of "liver macrophage" [28].

This review found sixty-seven papers that described the immunophenotype of human liver macrophages [21–23,31–94].

Physical and phenotypic description

KCs have also been defined by their peri-sinusoidal location in the liver lobule [95], and a morphological appearance that demonstrates a lack of the extensive fine cell membrane processes, usually found on dendritic cells. Scanning electron microscopy has demonstrated that KCs possess numerous lamellipodia, and show pronounced membrane ruffling [66]. They are described to classically lie tightly attached to the sinusoidal luminal surface [78]. Data are conflicting as to changes of KC density within the liver lobule; KCs have been described as being more densely populated in peri-central regions [36], but were also described as being diffusely pan-lobular, involving both the portal tracts and regions around the central vein [38].

Only a few cellular markers have been described in humans that are consistently expressed by KCs. CD68, a lysosome associated trans-membrane glycoprotein, involved in the metabolism of the low-density lipoprotein, is the most consistent reported marker for determining macrophage populations throughout the body and has been used throughout the literature to define macrophages within the liver. Subsequently the majority of papers (n = 37) used immunohistochemistry or fluorescence microscopy to describe the presence or absence of further cellular components (such as cell surface markers, or tissue distribution) on cells that also stain positive for CD68. Hence, a good working definition for a Kupffer cell in the literature to date is a peri-sinusoidal cell expressing CD68.

Heterogeneity

A number of papers report heterogeneity of KCs but to date there is no comprehensive definition of KC subsets. Morphological
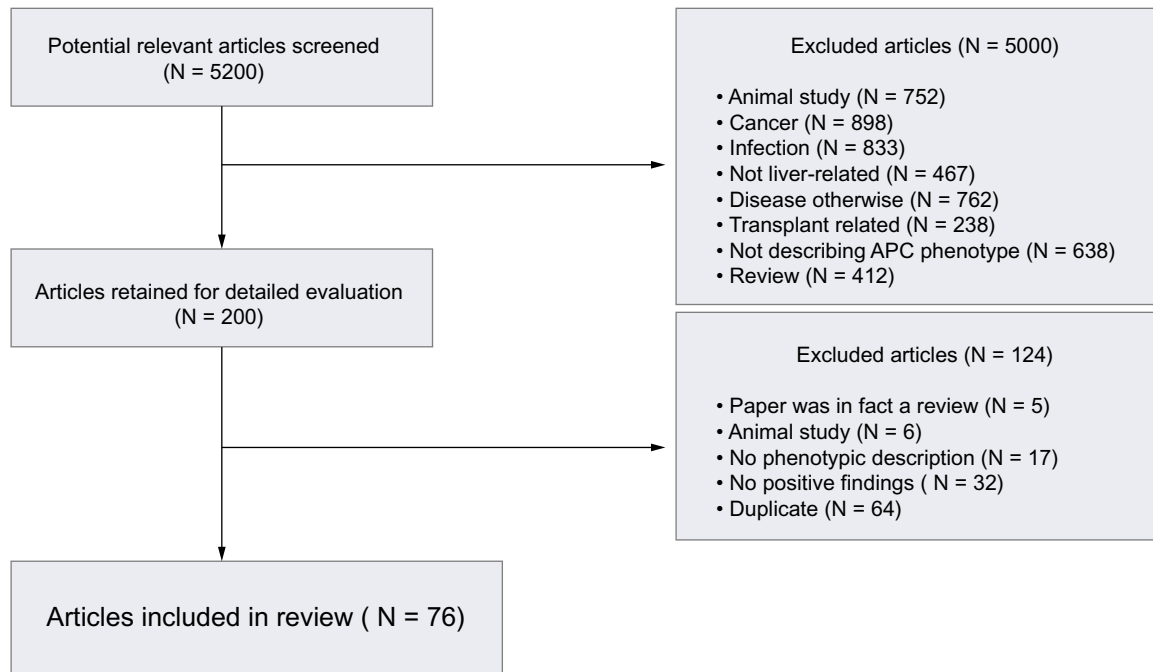
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Fig. 2. Quorum. Quorum diagram of results.

differences have been noted, such as macrophage populations with varying cell shapes in the portal compared to the central venous regions of the liver lobule [95], or two different populations of KCs being described as either "round" or thin" [32]. In 1995, Ueda *et al.* [86] described KC functional heterogeneity based on endogenous peroxidase activity that divided the cells into monocytes and four types of macrophages that showed a zonal distribution. These findings suggest that KCs are a collection of cells with varying phenotypes throughout different parts of the healthy liver, which is a concept that has been further supported by work in chronically hepatitis C virus infected livers [16].

The heterogeneous co-expression of other phenotypic markers by CD68+ cells supports KC heterogeneity. As noted above, some CD68+ cells co-express CD14, CD16, and CD163 [22] though it is not clear whether all these cells are resident macrophages, since activated monocytes can express all these molecules. Some KCs express Mac387, a marker for infiltrating macrophages as opposed to resident macrophages [42]. Similarly, CD68+ liver cells variably co-express a large range of APC markers, including lectins (CD209 [23], and CD299 or LSECtin [40]), complement receptors (predominantly CR1, CR3, and CR4 [70] and C5a (CD88) receptor [34]), Fc receptors (such as CD16, CD32, and CD64 [56]), and scavenger receptors (such as CD206 [40], CD163, and CD169 [69]). How the variable expression of all these markers in KCs relates to different locations within the liver lobule, and different cellular lineages, remains unclear.

Intriguingly, some CD68+ KCs in healthy liver express the proliferative marker, Ki67 [21], suggesting they are self-renewing *in situ*, rather than terminally differentiated cells derived from blood cells. In this context it is important to note recent evidence in mice (discussed below) for tissue macrophages that derive from the yolk sac and foetal liver rather than the bone marrow [29].

There is very little direct experimental evidence of the function of human KCs, so their roles are largely inferred from histology, electron microscopy, immunohistochemistry, and evidence from murine models.

Macrophages are classically not considered as potent as DCs at stimulating a T cell response, or as capable of travelling to drain secondary lymphoid tissue to instigate a systemic response [29]. However, KCs appear to be heavily involved in both the innate and the acquired immune responses within the liver. KCs express MHC class II, and express varying levels of co-stimulation markers (such as CD40, CD80, and CD86 [77]), as well as the inhibitory markers such as Z39Ig [89]. In the steady state they act as sentinel scavenging cells to process antigen from the gut. KCs readily phagocytose latex beads [96] and therefore play a major scavenging role in conjunction with the liver sinusoidal endothelial cells (LSECs) [33]. The migratory potential of KCs is still uncertain and although monocytes clearly express CCR2 and CX_3CR1, expression of chemokine receptors in $CD68^+$ cells is yet to be thoroughly explored [22].

Electron microscopy has shown KCs in direct contact with pit cells (liver NK cells), and liver stellate cells (the major collagen producing cell in the liver [78]). It is postulated that through this interaction they are key in instigating fibrosis following inflammation [93]. CD68+ KCs also co-express prokineticin 2/Bv8, a molecule strongly implicated in angiogenesis [43].

Liver dendritic cells
Ten papers describe the immunophenotype of liver dendritic cells (DC) in human liver [20,51,95,97–103].

Although the boundaries between macrophages and DCs have blurred in recent years, the major human DC subsets express well-defined cell surface markers that allow for their identification, using flow cytometry and multi-colour immunofluorescence

microscopy. Much of this work has been carried out using cellular markers common to DCs in blood and other non-hepatic tissue.

DCs in human blood are HLA-DR^{hi} cells that comprise 3 major non-monocytic subsets: plasmacytoid DCs (pDCs) expressing CD303 (BDCA2) and lacking CD11c; and two myeloid CD11c^{+} subsets – CD1c^{+} DCs expressing CD1c (BDCA1); and CLEC9A^{+} DCs expressing CLEC9A and high levels of CD141 (BDCA3) [25]. Peripheral and lymphoid tissues also have at least two myeloid CD11c^{+} migratory DC subsets (e.g. Langerhans cells) that express molecules such as CD207 and CD1a [27,104,105]. CD1c^{+} DC can secrete IL-10 in response to incubation with whole *E. coli* and the TLR-4 agonist lipopolysaccharide [106] and are therefore thought to have tolerogenic potential.

It is apparent that all three major classes of blood DCs are present in the liver, in addition to CD16+ monocytes [98], with CD1c+ DCs being the most prevalent subset [98]. The hepatic DC populations express similar markers to blood DCs, and when compared to skin and spleen DCs, liver DCs have an immature phenotype, with a relatively low expression of co-stimulatory molecules (CD40, CD80, and CD86), as well as maturation markers like CD83 [98,99].

Compared to blood, hepatic DCs were less efficient at antigen uptake, processing and presentation, including allo-stimulatory capacity, and upon TLR4 stimulation they secreted substantial amounts of IL-10, a cytokine associated with a tolerogenic phenotype [107]. Kwekkeboom *et al.* [108] found that compared to inguinal lymph node DCs, hepatic DCs were less capable of stimulating T cells, despite higher expression of HLA-DR, CD80, and CD86. Bamboat *et al.* found increased production of Foxp3^{+} Treg cells and IL-4 producing T cells (associated with the humoral immune response [109]) that were difficult to re-activate if initially activated by hepatic DCs in comparison to blood-derived DCs. Interestingly, liver DCs were also found to have a significantly decreased secretion of IL-12p70, which has been viewed as a pro-inflammatory cytokine [110].

Goddard *et al.* [99] used overnight migration to extract DCs from the liver. They found high HLA-DR, CD86, and CD11b prior to culture. They also found that these hepatic DCs produced larger amounts of IL-4 and IL-10 and lower levels of IL-12p70 compared to DCs from the spleen and skin. They report that the DCs extracted, using overnight migration, expressed low levels of CCR5 and were positive for the chemokine receptor CXCR4 and CCR7. Very little other work on intrahepatic liver DC chemokine receptors has been published.

In 2012 Haniffa *et al.* described the presence of CD141^{hi} DCs in the liver, using multi-colour flow cytometry, and also confirmed the presence of CD14^{+} APCs and CD1c^{+} DCs. CD141^{hi} DCs in other human tissues also express the definitive marker CLEC9A, and some reports indicate that these cells have some of the characteristics of murine CD8^{+} or CD103^{+} DCs [111], especially the ability to cross-present antigen to CD8+ T cells. However, no functional data from normal intrahepatic CD141^{hi} DCs have yet been published [112].

Further to studies of liver tissue from biopsy samples, liver perfusates from liver transplant procedures have described CD141 expressing DCs that appear to be of a more pro-inflammatory phenotype than CD14^{+} and CD1c^{+} MPS in the liver [102]. However, it is apparent that CD141 is much more widely expressed in liver cells than in blood, where it is largely restricted to CLEC9A^{+} DC [111]. Hence, CLEC9A^{+} DCs may only be a subset of the CD141^{+} cells, derived from human liver, so results from sorting on CD141 alone need to be interpreted with some caution [113].

While the majority of research on liver DCs has been to assess phenotype using flow cytometry, there is a small amount of histological work localising DCs, this has used light microscopy and immunohistochemistry. Myeloid DCs are predominantly located in the portal tract and periportal zones, with particular density around the bile ducts while pDC are found scattered throughout the liver lobule [114].

Discussion

Although similar, monocytes in the liver are different from the blood

As we noted, three major subsets of blood monocytes are considered to be present in liver, and it appears that liver monocytes are richer in CD16 expression than monocytes in the blood. CD16 monocytes probably derive from CD14 monocytes [115] and the increased number of CD16 monocytes may relate to the activation of classical monocytes in the liver. Clearly some cells bearing monocyte markers also express markers more commonly associated with KCs such as CD68 and CD163 [22]. Monocytes are therefore likely to be involved in transient inflammatory responses in the liver, but may also be precursor cells to some DC and KC populations, even in normal liver.

The literature does not define a organ resident or passenger MPS

The liver is a unique organ as it is highly vascular, blood filtering, and maintains a tissue resident population of the MPS. We currently lack markers that define cells that are transiently passing through the organ from those that are tissue resident. The fact that cells, bearing monocytic markers, can upregulate molecules more commonly associated with macrophages, such as CD68 and CD163, suggests that MPS precursors may alter their phenotype substantially as they traffic through the liver, and/or seed populations of KCs and DCs within the liver. Monocyte markers themselves are problematic: CD14 is also expressed by sinus-resident APCs in other human lymphoid organs [27], and also by endothelial cells [22], so it may not be specific for monocytes in the human liver; and CD14^{lo} "non-classical" monocytes, transiting through the sinusoids, may be difficult to distinguish from resident KC populations.

Analysis of c-Myb expression may be informative in this context. It was recently reported that the majority of tissue macrophages that persist into adulthood in mice appear to be negative for the transcription factor c-Myb, which is present on cells derived from haematopoietic stem cells [116]. A series of lineage tracking studies in mice have shown that the majority of tissue macrophages are not replenished from bone-marrow derived monocytes, but are self-replicating macrophages that seed the tissue from the yolk-sac and the foetal liver during embryogenesis [117]. Small numbers of macrophage populations are derived from monocytes under homeostatic conditions in the adult liver, with further mobilization of these cells only under inflammatory conditions [118]. Hence, it should now be feasible to identify markers, capable of unequivocally distinguishing between blood-derived MPS and those resident KCs that do not originate from a bone marrow precursor. Further to that, as in

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other tissue [26], analysis of transcription factors may improve this.

Several DC subsets are present in the liver

Liver DC subsets are more thoroughly described than liver macrophages, due to the more recent application of multi-parameter flow cytometry, detecting definitive cell surface markers.

The current evidence points to the liver containing similar DC populations as other tissue. All the major subsets found blood, including pDCs, CD1c+ DCs and CLEC9A+ DCs appear to be present. Unfortunately, functional data on these subsets are limited, as is a detailed expression of their phenotype (such as a complete description of the pathogen recognition receptors, CCRs, and lectins). The small amount of published data suggests that they are less mature than DCs found in blood and spleen, poorer at eliciting an antigen specific T cell response or MLR, and are associated with an increased production of IL-4 and IL-10 [98]. Of particular interest is the relative immunogenicity of CD141+ cells in both normal and diseased liver. Considering the increased expression of CD141 in tissue DC populations [119], it will be important to determine whether this is due to CD141^{hi}CLEC9A+ cells or any other liver cell that happen to express CD141.

The tolerogenic environment of the liver and the MPS

Although cells of the MPS are implicated in producing the tolerogenic environment of the liver, there remain large areas of MPS characterisation to be explored. However the limited data available from liver DCs suggest substantive differences in their ability to perform some functions, such as phagocytosis, migration, T cell stimulation, cross-presentation, and specific cytokine secretion.

Tolerance in the liver appears to be due to many factors, including the nature of T cells, the effect of other non-parenchymal cells, and large amounts of TGF-beta [120]. Production of IL-10 in response to bacterial cell wall components represents a prominent tolerogenic mechanism that appears to occur in all three groups of MPS cells in the liver. Considering the large amounts of bacterial cell wall products that enter the liver through the portal vein in the normal liver [121], this mechanism may be central to the liver's tolerant state. Studies of liver perfusates [122] have supported these findings and it appears that the loss of this mechanism in cirrhosis may be due to a modification of the MPS [123].

An area notably lacking data concerns the physiological response of the liver MPS to the presence of whole bacteria [121]. Translocation of bacteria and bacterial fragments from the gut is a common occurrence [11], and the responses they induce in different liver MPS subsets warrant further investigation as they presumably are part of the physiological role of some liver MPS subsets *in vivo*. In human blood, whole *E. coli* appears to promote a further induction of IL-10 expression by CD1c+ DCs [106] that is independent of TLR ligation; it is yet to be determined if this occurs in the liver, but may also be a further contributing factor in promoting hyporesponsiveness.

The liver DCs appear to have a relatively immature phenotype with a lower expression of co-stimulatory markers [98]. This, coupled with their inferior abilities of antigen uptake, processing and presentation, may be contributing factors to their decreased ability to simulate T cells [98,99]. When T cells are primed by liver DCs they are then more difficult to re-activate compared to blood DCs (this T cell hyporesponsiveness appears to be partially regulated through an IL-10 dependant mechanism), and are more likely to be Foxp3+ regulatory T cells [98]. These are all factors that may contribute to a tolerogenic environment in the liver.

Kupffer cells appear to be heterogeneous

In general, KCs harbour many of the characteristics of other tissue macrophages. They readily phagocytose latex beads [96] and express a variety of macrophage related scavenger receptor molecules [40,69]. However, there is clearly variability in the expression of these markers and other molecules amongst KCs and light [32] and electron [86] microscopy findings have identified that KCs are composed of differing subsets of cells, which are distributed through different zones of the liver lobule. These findings indicate that KCs appear to be heterogeneous, but exploration of KC subset composition and function is lacking.

Through their expression of molecules involved in the presentation of antigen to T cells (MHC class II, T cell co-stimulatory molecules) it seems likely that at least some KCs are involved in presenting antigen to liver-resident T cells but their migratory potential and their capacity to traffic antigen to lymph nodes remain uncertain.

Macrophages are particularly difficult cells to isolate from human liver, due to the loss of large numbers of cells during isolation and lack of consistent cell-membrane markers that can be used in cell sorting. CD68 has a variable expression on the cell surface [124], and little else beyond CD14, CD16, and CD163 has been considered, despite the obvious overlap with transiting monocytes [22,125].

There is therefore a need to define and accurately sort KC subsets, in order to enable functional assays for studies in both healthy liver and disease. Multicolour fluorescence microscopy and flow cytometry may provide sufficient details to accurately and comprehensively assess a molecular phenotype for KCs (as has been the case with characterizing other cell subsets [126]). As our understanding of the complexity of macrophages increases, the limits of general terms, such as "Kupffer cell" [127], may make it timely to review and revise our terminology.

Liver microanatomy

Ultimately, understanding MPS function in the liver will require an appreciation of histological differences in the distribution of these different subsets throughout the different areas of the liver and an improved understanding of their role and interaction with sinusoidal endothelia, stellate cells and other leukocytes. Despite data indicating other cell types, such as hepatocytes [128] and LSECs [129,130] that are different in different zones of the liver, little data exist beyond gross morphological observations for zonal distributions of different MPS populations, especially KCs.

Although it is apparent that KCs are scattered throughout the liver lobule, without sophisticated techniques to determine subsets, based on molecular marker expression, using more than one marker (such as CD68) it is impossible to ascertain any accurate intralobular differences in subset distributions, or examine how MPS function changes with location. It would be reasonable for the composition and function of perisinusoidal KCs to also change across these zones as observed with hepatocytes [128] and LSECs [129,130]. KCs are closely associated with the sinusoidal

endothelia; and are likely to be able to interact extensively with the slow moving plasma and blood cells as they transit [11].

In contrast, the portal tract is where the majority of the liver's dendritic cells reside [6], and it becomes very heavily populated with leukocytes during liver inflammation [131]. The portal tract also houses the lymphatic endothelium, and is hence the conduit through which the MPS will travel to draining lymph nodes. As a result of these features it is thought to be the area of the liver lobule where the majority of antigen presentation to T cells occurs. A thorough understanding of the relationship of each MPS subset to the micro-anatomical structure of the liver will therefore help to inform knowledge of their function.

Clinical significance

In the case of transplantation, despite showing superior graft acceptance in comparison to other transplanted organs, graft rejection is still a major problem in the liver transplant setting with rejection rates as high as 20–40% [132]. The focus of the current therapy is primarily to reduce the presence of a pro-inflammatory state at the time of transplantation through the use of calcineurin inhibitors, such as cyclosporin or tacrolimus in combination with cytolytic agents, such as mycophenolate or azathioprine [3]. These drugs are associated with significant side effects, directly through renal and cardiac toxicity but also through the indirect effects of inducing a broadly dysregulated immunological state, leading to higher rates of cancer, infection, and *de novo* autoimmune disease [133]. The study of MPS in the liver will therefore improve our understanding of the liver's tolerogenic state and the nature of the biological processes, involved in the loss of this normal state. Understanding which subsets are most tolerogenic or immunogenic may identify targets for up- or downregulation, depending on the desired tissue response.

Further to this, appreciating which subsets are most capable of antigen presentation and T cell stimulation allows for the opportunity to improve the efficacy of immunotherapy directed towards cancer and infectious diseases. Primary liver cancers, such as hepatocellular carcinoma (HCC), and metastasis (for example from colon cancer), have a high mortality rate and continue to pose huge burdens on the medical community [134]. HCC in particular reflects the consequences of long-term liver inflammation through insidious disease and infection, such as hepatitis C and hepatitis B, both of which place a huge burden on global health [135].

Conclusion

Currently strategies to manipulate the liver MPS are impaired by a lack of appreciation of the populations of monocytes, macrophages and DCs present in human liver, and their functional attributes. Modern techniques, already being used to describe MPS populations in other organs, should now be implemented to improve the understanding of the liver MPS. An improved and more accurate understanding of these cells will be vital for the accurate description of cell function, and elucidation of appropriate targets for therapy. This will cover a range of applications from enhancing immunity to cancer or infectious agents, to inducing and maintaining tolerance, such as in liver transplantation and autoimmune disease.

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