Hepatology Snapshot

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Dendritic cells in liver injury and fibrosis: Shortcomings and promises

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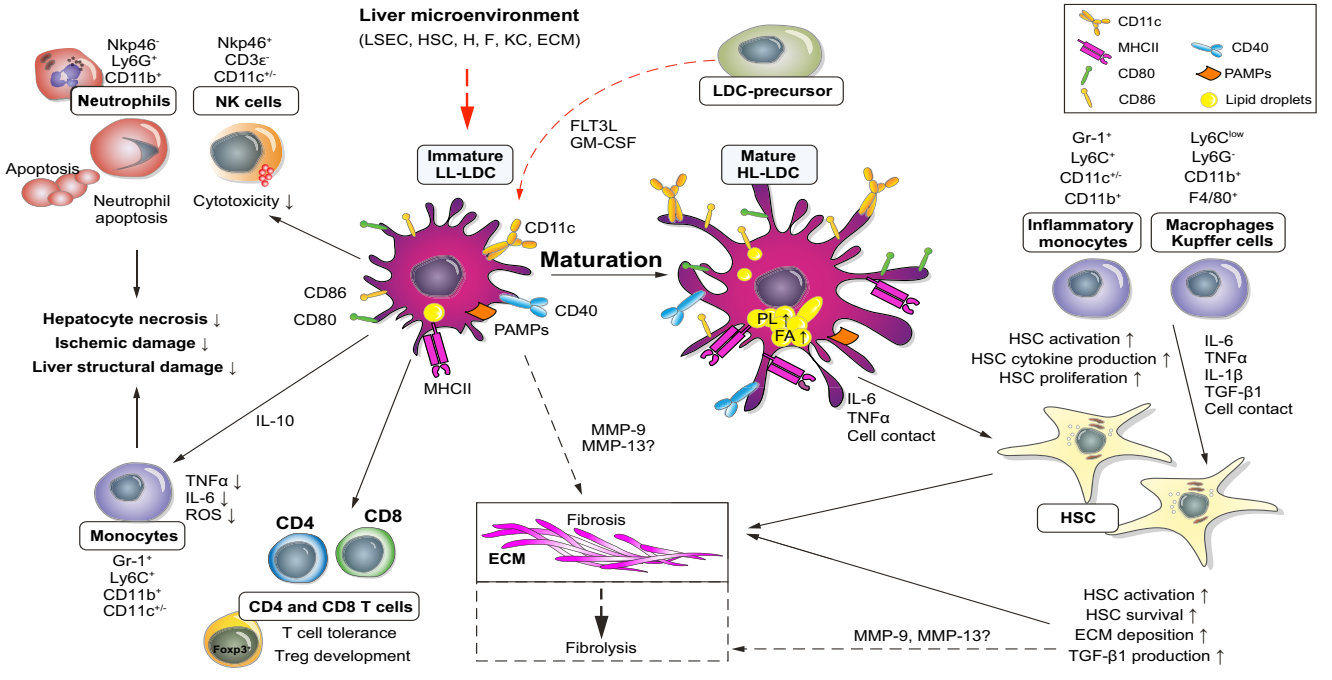
Key facts

The phenotype and function of liver dendritic cells (LDCs) are poorly understood. This Snapshot summarizes our current knowledge on LDCs in the healthy and injured liver, and their role in fibrosis progression and reversal. It also draws attention to various pitfalls in the current experimental design and conclusions based on available data.

A

Mouse LDC subsets	CD103⁺ DC	CD103- DC	pDC	LDC precursor
	CD45⁺ Lin⁻ PDCA-1⁻ CD11c⁺ MHCII⁺ CD103⁺ CD11b⁻	CD45⁺ Lin⁻ PDCA-1⁻ CD11c⁺ MHCII⁺ CD103⁻ CD11b⁺	CD45⁺ Lin⁻ PDCA-1⁺ CD11c⁺	CD45⁺ Lin⁻ MHCII⁻ CD11c⁺ SIRP1a^{low}
Specific features attributed to DC subtypes	Resemble CD8+ lymphoid tissue resident-DC	Heterogeneous, contains cells derived from both the DC and monocyte lineage	Type-I IFN production and antiviral response	*In situ* LDC development and homeostasis
Transcription factor involved in murine LDC development	BaQ3 Id2 FLT3L IRF8	Independent from M-CSF ?	IRF8 E2-2 Id2	
Human LDC subsets	**BDCA3+ DC**	**BDCA1+ DC**	**pDC**	**LDC precursor**
Human LDCs	CD45⁺ HLA-DR⁺ CD141/BDCA-3⁺ CD123⁻ CD11c⁺ CD14⁻ XCR1⁺	CD45⁺ HLA-DR⁺ CD1c/BDCA-1⁺ CD123⁻ CD11c⁺ CD141/BDCA-3⁻ CD14±	HLA-DR⁺ CD123⁺ CD11c⁻ CD45RB⁺ CD303/BDCA-2⁺ CD304/Neuropilin-1/ BDCA-4⁺	?

B



Conclusion

LDCs are immature and tolerogenic in the steady state. During chronic liver injuries a pro-inflammatory LDC population prevails, while in acute liver damage LDCs appear to be protective, preventing structural damage.

Keywords: CD11c-DTR; Flt3L; Tolerance; Liver dendritic cells.
Received 12 April 2013; received in revised form 21 May 2013; accepted 22 May 2013

Summary

The phenotype and function of liver dendritic cells (LDCs) are poorly understood. This Snapshot summarizes our current knowledge on LDCs in the healthy and injured liver, and their role in fibrosis progression and reversal. It also draws attention to various pitfalls in the current experimental design and conclusions based on available data.

LDC populations

DCs are the major APCs and link innate and adaptive immunity. LDCs localize within the portal area of the healthy liver and sparsely in the parenchyma [1]. The population of LDCs is heterogeneous and three murine LDC subsets can be distinguished: $CD103^+$, $CD103^-$, and pDCs [1,2] (panel A, figure). LDC development is regulated by transcription factors, which mediate DC-development in lymphoid and other non-lymphoid organs [2,3]. The liver contains DC precursors from which LDCs can be expanded *in situ* via GM-CSF or FLT3L [1,2]. Our current understanding of LDCs is largely based on the evaluation of either $MHCII^+$ myeloid or $CD11c^+$ cell populations, only part of which may be true LDCs. Less is known about the role of LDCs in humans.

LDCs as mediators of tolerance

$CD11c^+$ cells from the healthy liver display a low capability to endocytose antigens and to stimulate T cells, but produce increased IL-10 compared to their splenic counterpart [1]. This tolerogenic phenotype appears to help maintain allograft survival after liver transplantation [1]. Notably, the hepatic microenvironment promotes regulatory DC development, and likely educates circulating DCs as they translocate from the blood to the lymph via the hepatic sinusoids [1]. LDCs generated from LDC precursors demonstrate low expression of co-stimulatory molecules and are resistant to conventional maturation stimuli (LPS, TNFα) [1], consistent with their immature phenotype despite constant exposure to gut-derived bacterial products. Instead, LDCs mature after attachment to ECM (e.g., fibronectin, collagen-type I, laminin) [1] *in vitro*, yet the relevance *in vivo* is unknown. A valuable classification of LDCs exists according to their lipid content [4]. HL-LDCs, displaying elevated triglycerides and phospholipids due to their acetyl-CoA carboxylase activity, can mount a strong CTL response, while LL-LDCs (low triglycerides, phospholipids) are less mature and can induce oral tolerance [4]. Liver pDCs, different from LL-LDCs, can also mediate oral tolerance, suggesting the involvement of multiple LDC subsets [1].

Pro-inflammatory LDCs in liver injury

In the models of MCD-diet-induced NASH and TAA-induced fibrosis, HL-LDCs were the dominating hepatic DC species, characterized by increased TNFα production, FA synthesis, and endoplasmic reticulum stress [4]. Surface marker analyses of HL-LDCs revealed the involvement of multiple LDC subsets [4]. Increased numbers of monocyte-derived $CD11c^+$ cells were observed in biliary fibrosis (BDL), with higher capacity to acquire antigen and stimulate T cells [1,2]. $CD11c^+$ cells were elevated up to 7-fold in fibrosis induced by TAA and recombinant leptin, producing excess IL-6, TNFα, and activating HSCs through TNFα and direct cell-contact [5]. $CD11c^+$ cell depletion reduced proinflammatory cytokine expression by liver non-parenchymal cells, supporting their proinflammatory role in established fibrosis [5]. Notably, during fibrosis progression, $F4/80^+$ macrophages and not $CD11c^+$ cells promoted survival of HSCs via NF-κB in both the BDL and CCl_4 fibrosis models [6]. HSC survival was dependent on IL-1 and TNFα production by $F4/80^+$ macrophages and only marginally by LDCs. Repetitive depletion of $F4/80^+$, but not $CD11c^+$ cells, ameliorated fibrosis progression, thus proinflammatory LDCs seem to be dispensable for excess ECM deposition in these models.

Resident LDCs as guardians of liver integrity

In acetaminophen-induced acute liver injury, depletion of $CD11c^+$ cells exacerbated liver pathology [7]. Here expansion of the $CD11b^+CD11c^+$ population induced neutrophil apoptosis, inhibited NK cell cytolytic activity and decreased hepatocyte necro-apoptosis [7]. Moreover, during ischemia-reperfusion, IL-10 producing $CD11c^+$ cells suppressed proinflammatory cytokines and ROS produced by freshly recruited inflammatory monocytes [1,8].

LDCs exhibit fibrolytic properties and depletion of $CD11c^+$ cells after withdrawal of CCL_4 led to slower fibrosis regression and reduced clearance of activated HSCs [9]. Accordingly, *in vivo* expansion of LDCs by Flt3L promoted fibrolysis after withdrawal of CCl_4, partly via production of MMP-9 [9]. Another study described the accumulation of $CD11b^+F4/80^+$ myeloid cells during CCl_4-induced fibrosis. Their depletion in the CD11b-DTR mouse model which eliminates macrophages and a subpopulation of LDCs, retarded spontaneous fibrosis regression, with an important role played by MMP13 [10]. Using the same model, a subpopulation of these fibrolytic $CD11b^+F4/80^+$ cells were recently characterized as MMP9 expressing Ly6Clo monocyte-derived macrophages [11]. Due to overlapping surface markers among monocytes, macrophages and DCs, it remains unclear to what extent LDCs contribute to fibrolysis besides the better defined role of macrophages.

Novel possibilities for studying LDCs

The CD11c-DTR model has been the most widely used animal model in LDC biology. DT treatment of BM-chimeras (CD11c-DTR BM transferred to irradiated wild type mice) results in the depletion of LDCs. However, this treatment also affects $CD11c^+$ macrophages, monocytes, NK cells, and pDCs [12]. A novel, yet understudied model is the zbtb46-DTR mouse [13], based on a transcription factor that is expressed only in DCs and DC-committed precursors. Thus, deletion of $zbtb46^+$ cells appears to spare non-DC $CD11c^+$ cells, which could help better delineate the effect of LDCs observed in the CD11c-DTR model. Furthermore, a model is needed that would permit constitutive DC depletion.

The field of DC biology in *human* liver diseases is even less advanced, and differentiation of human DCs from other myelomonocytic cells has been difficult to achieve [1]. As in rodents, the properties of resident human LDCs are expected to be highly dependent on the liver microenvironment [1,14], and due to the variant cell surface markers, conclusions cannot simply be transferred from mice to humans and *vice versa* (panel A, figure).

Fig. (A) Dendritic cell subsets. Comparison of the distinguishing surface markers and specific features of the various LDC subsets. Three murine LDC subsets ($CD103^+$, $CD103^-$, and pDC) and their putative human counterparts ($BDCA\text{-}1^+$, $BDCA\text{-}3^+$, and pDC) can be distinguished in the liver. It is suggested, but not entirely proven yet, that many features of murine DCs can be directly translated to the corresponding human DC subsets.
(B) Multiple roles of LDCs in the healthy and injured liver. LDCs are immature and tolerogenic in the normal steady state liver. LDC precursors within the liver give rise to resident LDCs. The liver microenvironment (especially fibroblasts, mesenchymal stem cells and their ECM) supports tolerogenic/immature LDC differentiation. During acute liver injury, resident LDCs protect liver integrity, e.g., via production of IL-10, induction of neutrophil apoptosis and downregulation of NK-cell cytotoxic activity. During chronic liver injury, mature pro-inflammatory LDCs dominate the liver. These LDCs contain more lipid droplets and exhibit increased fatty acid and phospholipid synthesis due to their acetyl-CoA carboxylase activity. They release various cytokines, which result in inflammatory monocyte recruitment and HSC activation. LDCs can produce MMP-9 that appears to be central to removal of excess ECM during fibrosis regression. F, fibroblasts; H, hepatocyte; HSC, hepatic stellate cell; KC, Kupffer cell; LSEC, liver sinusoidal endothelial cell; PAMPs, pathogen-associated molecular patterns.

Abbreviations: APC, antigen presenting cell; BM, bone marrow; BDL, bile duct ligation; DCs, dendritic cells; Batf3, basic leucine zipper transcription factor, ATF-like 3; CCL4, carbon tetrachloride; CTL, cytotoxic T lymphocyte; DT, diphtheria toxin; DTR, diphtheria toxin receptor; ECM, extracellular matrix; FA, fatty acid; Flt3L, FMS-like tyrosine kinase 3 ligand; GM-CSF, granulocyte macrophage colony-stimulating factor; HSC, hepatic stellate cells; HL-LDC, high-lipid liver dendritic cell; Id2, inhibitor of DNA binding 2; IL-6, interleukin-6; LDC, liver dendritic cell; LL-LDC, low-lipid liver dendritic cell; LPS, lipopolysaccharide; MCD, methionine choline deficient; MCP-1, monocyte chemotactic protein-1; MMP-9, matrix metalloproteinase-9; MMP13, matrix metalloproteinase-13; NASH, non-alcoholic steatohepatitis; PAMPs, pathogen-associated molecular patterns; PBMC, peripheral blood mononuclear cells; pDC, plasmacytoid dendritic cell; TNFα, tumor necrosis factor-alpha; TAA, thioacetamide; zbtb46, zinc finger and BTB domain containing 46.

Conclusions

LDCs are immature and tolerogenic in the steady state. During chronic liver injuries a pro-inflammatory LDC population prevails, while in acute liver damage LDCs appear to be protective, preventing structural damage (panel B, figure). In many studies, the employed surface markers did not permit a differentiation of LDCs from e.g., monocytes, macrophages or other myeloid cells, stressing the need to use better surface markers and transgenic tools for studying DC biology in the liver.

Financial support

This work was supported by the Alexander von Humboldt Foundation Sofja Kovalevskaja Award to VLK. DS received funding from the NIH, the European Union, the Research Center of Immunology Mainz, the State of Rhineland-Palatinate, the German Research Association (DFG) and the German Ministry of Education and Research.

Conflict of interest

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

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