

Dendritic cells in NASH: Friend or foe?

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Article commented:

Henning JR, Graffeo CS, Rehman A, Fallon NC, Zambirinis CP, Ochi A, Barilla R, et al. Dendritic cells limit fibro-inflammatory injury in NASH. *Hepatology* 2013. Doi: 10.1002/hep.26267 [Epub ahead of print].

Comment:

Nonalcoholic fatty liver disease (NAFLD) may be the most common chronic liver disease in the world.¹ The spectrum of NAFLD ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma.² NAFLD is often associated with obesity, insulin resistance and metabolic syndrome.^{1,2} Obesity accompanies inflammation and activation of immune signaling pathways which could lead to hepatic injury and NASH.^{1,2} Recent studies have illuminated the role for immune cells in the pathophysiology of NASH,³ for example, the role of the TNF- α produced by the resident hepatic macrophages (Kupffer cells)⁴ and function of natural killer cells (NK) in preventing fibrosis.⁵ A novel study by Henning, *et al.*, which appeared in a recent issue of *Hepatology*,⁶ attempts to delineate the role for yet another immune subset, namely the dendritic cell (DC) in NASH.

Dendritic cells are positioned at the threshold between innate and adaptive responses.⁷ They are a heterogeneous cell-type that promotes both pro-inflammatory and anti-inflammatory immune responses.⁸ DCs, based on their subtype and activation status, elaborate an array of cytokines such as IL-6,

IL-12, TNF α and IL-10.⁷ In addition, DCs as professional antigen presenting cells (APC) play an important role in initiating adaptive immune responses by processing and presenting antigen to CD4 T cells and CD8 T cells.⁹

Henning, *et al.* used a combination of *ex vivo* and *in vivo* phenotypic and functional analyses to clarify the role of recruited dendritic cells in the pathogenesis of NASH by using the methionine-choline deficient diet (MCD) rodent model of NASH.¹⁰ They found that CD11c+ MHC+ DCs expanded in number early in the NASH liver to 3-4 times the levels found in control mice and underwent activation and maturation as measured by up-regulation of the cell-surface co-stimulatory markers, CD40, CD54, CD80 and CD86. Concomitant with the increase in the DCs; the proportions of other CD45+ (a marker for all leukocytes) cell-populations such as Kupffer cells, monocytes, neutrophils and CD8 T cells also increased, while others such as natural killer cells (NK), natural killer T cells (NKT) cells, CD4 T cells and B cell numbers underwent a diminution. The authors confirmed that the amplification of DCs was specific to the liver, with no evidence of splenic DC number or phenotype alteration.

Consistent with their *in vivo* phenotype, NASH DC (DC isolated from NASH mice after 6 weeks on a MCD diet) when cultured *in vitro*, also displayed inflammatory characteristics, such as increased production of IL-6, TNF α and MCP-1 and increased IL-6 and IFN γ production upon TLR-9 stimulation. Furthermore, utilizing *in vitro* co-culture experiments between DC and different classes of T cells, the authors demonstrated that NASH DC augmented the proliferation of CD4 T cells and increased their ability to produce Th1, Th2 and Th17 cytokines, whereas they had no effect on CD8 T cells.

Henning, *et al.* followed up the *in vivo* and *in vitro* phenotypic and functional analyses with compelling *in vivo* DC depletion studies to fully explore the role of DC in NASH. In these experiments, the authors used bone-marrow chimeric CD11c.DTR mice, wherein CD11c is only expressed in bone-marrow

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derived cells and the diphtheria toxin (DT) receptor (DTR) is expressed under the CD11c promoter. In these mice, DT injection results in elimination of CD11c expressing cells. Although this method has potential complications related to the fact that CD11c is not uniquely expressed on DCs, but is also present on the macrophages and CD8 T cells¹¹ and the diphtheria toxin has toxicity effects,¹²⁻¹⁴ this system remains a viable method to reveal the role of CD11c positive cells *in vivo*.^{12,13}

Using chimeric CD11c.DTR mice, the authors depleted CD11c expressing cells and demonstrated that their elimination leads to exacerbation of the NASH phenotype, suggesting a regulatory role for DC in NASH. They found that the number of regulatory T cells (Tregs) and CD4 T cell numbers diminished whereas CD8 T cells expanded. There was an increase in the hepatic inflammatory cell infiltrate and inflammatory cytokine and chemokine production in the NASH liver of mice depleted of DC. In contrast, they observed decreased levels of IL-10, a regulatory cytokine. Kupffer cells, monocytes and neutrophils were activated to produce higher levels of IL-6, TNF- α and IL-1 β . Kupffer cells expressed higher levels of TLR-4 and -9 in the NASH livers devoid of DC. Moreover, the authors found that upon DC ablation, levels of apoptosis increased in the NASH livers- with increased levels of apoptosis markers, PAR4 and cleaved caspase-3. There was also increased gene expression of mediators of apoptosis such p53, FasL and Bcl2, suggesting that one of the mechanisms by which DCs regulate NASH is by clearance of apoptotic bodies. In addition to increased inflammation and apoptosis, the NASH (-DC) liver exhibited enhanced fibrosis as indicated by histologic evidence of fibrosis and gene expression of fibrogenesis markers such as TIMP1, collagen 1 α 1, TGF- β and MMP9. DC depletion only in the context of NASH was associated with exacerbation of disease but not in the LPS treated mice on a normal diet or control mice, suggesting a specific immunomodulatory role for dendritic cells in the diseased liver.

Finally, Henning and co-workers demonstrated that DCs are not only involved in limiting inflammation and fibrogenesis during the progression of NASH, but are also critical for the resolution of NASH. When CD11c+ DCs were ablated during the recovery phase of NASH (achieved by putting the MCD-fed animals back on a control diet), the resolution of inflammation and fibrosis was delayed markedly, suggesting that dendritic cells are involved in the disease recovery phase.

Although this study does not address the source of dendritic cells in the liver, and utilizes a dietary rodent model that is different from human NASH,¹⁰ this study clearly demonstrates that DC are dynamic cells that contribute to maintaining an anti-inflammatory state in the NASH liver in an otherwise highly inflammatory milieu. Other questions about the effect of DC on the relative contribution of the anti-inflammatory modulators, IL-10 and Tregs in achieving this status remain to be answered. Identification of the complex signals which regulate such anti-inflammatory pathways could reveal potential therapies aimed at eliciting the anti-inflammatory nature of hepatic DC as a means to ameliorate the deleterious effects of NASH.

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