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Eating Macrophages for A Healthy Anti-NASH Meal

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Summary

Nonalcoholic steatohepatitis (NASH) is a common liver disease involving interactions between a variety of liver cell types. In this issue of Immunity, Wang et al. show that efferocytosis of dying lipid laden hepatocytes by hepatic macrophages protects against the development of NASH.

Non-Alcoholic Fatty Liver (NAFL) is characterized by lipid accumulation in hepatocytes creating steatosis with mild inflammation. An estimated 25% of patients with NAFL will go on to develop Non-Alcoholic Steatohepatitis (NASH) which involves not only steatosis but hepatocyte damage (ballooning), substantial intrahepatic inflammation and hepatic stellate cell-mediated fibrosis. Within the liver, hepatocytes, Kupffer cells (KCs), monocyte-derived recruited hepatic macrophages (RHMs), and hepatic stellate cells (HSCs), all participate in an intra-organ communication system which eventually leads to the classic findings of NASH, i.e. steatosis, inflammation and fibrosis (Schwabe et al., 2020). However, since hepatocyte steatosis is the first abnormality detected, much interest has been paid to the role of steatotic hepatocytes in promoting the full NASH syndrome. One idea is that toxic lipids are produced by hepatocytes which can drive inflammation and perhaps fibrosis, although such a lipid has not been identified despite many years of research.

Over the past few years, triggering receptor expressed in myeloid cells 2 (TREM2) on hepatic macrophages, including RHMs and KCs, has emerged as an important cell surface molecule with protective effects against NASH development. Thus, several papers have shown that genetic ablation of TREM2 promotes the development of NASH in mice placed on typical NASH-inducing diets (Hendrikx et al., 2022; Hou et al., 2021). Likewise TREM2 has also been implicated in the etiology of NASH in humans (Indira Chandran et al., 2022). TREM2 is a member of the immunoglobulin superfamily of cell surface receptors (Deczkowska et al., 2020). TREM2 is predominantly expressed in myeloid cells and binds to various lipid species and proteins. Although TREM2 has a relatively short intracellular domain, it can transmit intracellular signals by interacting with adaptor proteins, such as DNAX activation protein 12 (DAP12) and DAP10. In liver, the number of $Trem2^{+}$ macrophages increases during the development of NASH. Through unknown mechanisms, stimulation of surface TREM2 can promote intracellular signaling programs in macrophages that protect against the conversion of NAFL to NASH (Hou et al., 2021). It is also

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known that the extracellular domain of TREM2 can be actively degraded by liver matrix metalloproteinases creating a soluble form of TREM2 (sTREM2) which is elevated in the blood of both NASH mice and NASH patients (Feuerbach et al., 2017; Wunderlich et al., 2013). sTREM2 may prevent the normal TREM2 ligands from effectively stimulating full length TREM2, facilitating conversion to NASH. sTREM2 levels in the blood of mouse and NASH patients are elevated and this may also serve as a biomarker for this disease (Hendrikx et al., 2022; Indira Chandran et al., 2022).

Since TREM2 can act as a lipid receptor, it has been proposed that Trem2^{+} macrophages are lipid scavenging phagocytes. However, the effect of TREM2 on this function of macrophages is not clearly understood. Wang et al. performed a comprehensive study on the expression and function of TREM2 in liver macrophages during the development NASH. Efferocytosis refers to the phagocytic engulfment of a cellular corpse. The study revealed that TREM2 was necessary for efferocytosis of steatotic hepatocytes by liver macrophages and the decline of TREM2 protein expression at the onset of NASH enhanced NASH development by causing the accumulation of dead hepatocyte debris which promoted inflammation. Consistent with previous reports (Seidman et al., 2020), Wang et al found that *Trem2* mRNA expression was gradually increased in liver macrophages during the transition from NALF to NASH, in both mice and humans. This was due to increased release of sphingosine-1-phosphate (S1P) from dying hepatocytes. S1P activated its cell surface receptor S1PR1, but not S1PR2, on macrophages to increase *Trem2* mRNA expression. Upon *in vitro* treatment with palmitic acid, hepatocyte sphingosine kinase gene (*Sphk1* and *Sphk2*) expression was increased with enhanced release of S1P. Treatment with S1P directly increased *Trem2* expression in bone marrow-derived macrophages (BMDMs), whereas, siRNA silencing of *S1pr1* or pharmacological inhibition of S1PR1 and 3 reduced the ability of apoptotic AML12 hepatocyte-conditioned medium to induce *Trem2* expression in BMDMs. Moreover, S1PR1 and 3 inhibitor treatment reduced *Trem2* expression in the liver of mice fed a NASH-inducing diet. In addition, the authors revealed that siRNA silencing of *SphK1* and *Sphk2* in AML12 hepatocytes blocked the effect of these cells to increase macrophage *Trem2* expression. On the other hand, unlike mRNA, TREM2 protein expression was only transiently increased in humans and mice with simple steatosis, and declined at the onset of NASH. Wang et al found that this was due to TNFα and IL-1β-induced ADAM17 expression and activity, which cleaves membrane-bound TREM2. This occurred without changes in the amount of *Trem2* transcript. Indeed, ADAM17 protein expression was increased in the liver of NASH mice, but not in mice with simple steatosis. Moreover, *ADAM17* mRNA expression was higher in the liver of NASH patients. Among hepatic macrophage subtypes, they showed that membrane-bound Trem2 was more abundantly expressed in TIM4^{-} CLEC4F^{-} RHMs compared to KCs. Interestingly, genetic ablation of myeloid-specific (*Lyz2*-Cre) *Trem2* did not change the number of TIM4^{-} RHMs in mice fed a NASH-inducing diet. When assessed in BMDMs, genetic ablation of *Trem2* reduced the expression of genes involved in phagocytosis without changes in the expression of genes involved in lipid metabolism or inflammatory pathways. Consistent with this, they found that genetic ablation of *Trem2* in BMDMs or liver macrophages reduced efferocytosis of apoptotic AML12 hepatocytes compared to WT cells. Moreover, treatment with anti-TREM2 antibody or the soluble form of TREM2 (which can act as a

decoy receptor for TREM2 ligands) blocked efferocytosis of apoptotic AML12 hepatocytes by WT BMDMs. Consistent with these results, myeloid lineage-specific *Trem2* deficient mice developed a more severe NASH phenotype on a NASH-inducing HFD with increased number of TUNEL-positive apoptotic cells and activated caspase-3 expression in liver. Feeding a HFD did not cause NASH in WT mice. However, genetic ablation of myeloid *Trem2* allowed the development of a NASH phenotype, including a greater number of apoptotic cells in liver of mice on a 60% HFD. This occurred without changing the amount of liver triglyceride and cholesterol and the expression of lipid metabolism genes. While it is known that that the phagocytic activity of KCs in NASH is impaired (Gao et al., 2022), this is the first study that measures the specific function of efferocytosis by liver macrophages. This study does not reveal whether it is the KCs or recruited monocytic macrophages (RHMs), which are the major contributors to this deficit in efferocytosis, but, they do show that liver macrophages in general demonstrate this defect. There currently are methods for separating out KCs vs. RHMs to reach definitive conclusions on this point. Wang et al also found that administration of the soluble form of TREM2 did not rescue the NASH-prone phenotype of myeloid Trem2-deficient mice, indicating that the protective effects of TREM2 were largely conferred by its membrane-bound form. Together these results provide additional mechanisms for how TREM2 protein and mRNA expression is regulated in liver macrophages and how membrane-bound Trem2 promotes efferocytosis of large, lipid filled, dying hepatocytes and protects against the development of NASH.

While this paper is an important study taking the field forward, there are several important questions it raises for the future. In the absence of adequate TREM2 function, dying steatotic hepatocytes accumulate and propagate the development of NASH. However, what is the mechanism? Does this require the release of a putative toxic lipid. Do these non-engulfed hepatocytes promote inflammation and do they signal directly to HSCs to promote fibrosis or is it the enhanced inflammatory tone that stimulates fibrogenesis. Would TNFα and IL-1β administration in NAFL promote Adam17-mediated TREM degradation and would treatment with IL-1β and/or TNFα inhibitors prevent progression to NASH? What is the main source of TNFα and IL-1β which induce membrane-bound TREM2 degradation? What is the cause of resident KC cell apoptosis under NASH conditions? What factors stimulate TREM2 to promote efferocytosis. And finally, is the TREM2 mediated efferocytosis a property of recruited monocyte derived macrophages, KCs, or both.

These studies provide an important advance, placing deficient efferocytosis as an important mechanism in NASH development. Thus, eating a fatty meal in the form of lipid-laden hepatocytes has a healthy side with respect to NASH development.

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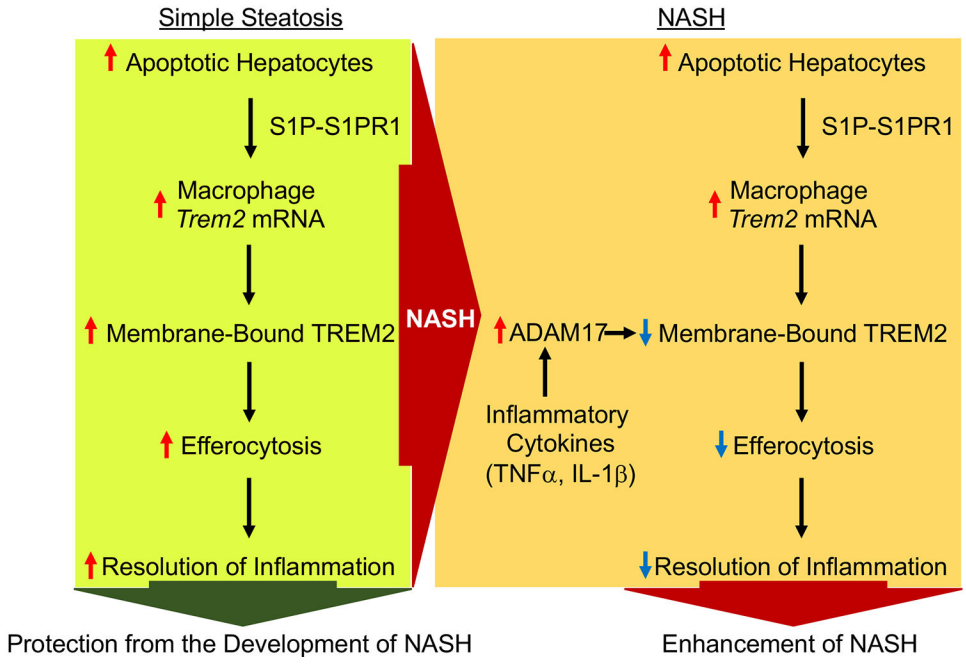

Figure 1.

Regulation of TREM2 expression in hepatic macrophages and the effect of membrane-bound TREM2 on efferocytosis of steatotic hepatocytes and the development of NASH (Wang et al.)