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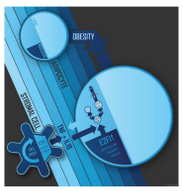

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LC3-associated phagocytosis in myeloid cells, a fireman that restrains inflammation and liver fibrosis, via immunoreceptor inhibitory signalling

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Abstract

Control of systemic and hepatic inflammation, in particular originating from monocytes/macrophages, is crucial to prevent liver fibrosis and its progression to end-stage cirrhosis. LC3-associated phagocytosis (LAP) is a non-canonical form of autophagy that shifts the monocyte/macrophage phenotype to an anti-inflammatory phenotype. In a recent study, we uncovered LAP as a protective mechanism against inflammation-driven liver fibrosis and systemic inflammation in the context of cirrhosis. We observed that LAP is enhanced in blood and liver monocytes from patients with liver fibrosis or those who progress to cirrhosis. Combining studies in which LAP was pharmacologically or genetically inactivated, we found that LAP limits inflammation in monocytes from cirrhotic patients, and the hepatic inflammatory profile in mice with chronic liver injury, resulting in anti-fibrogenic effects. Mechanistically, LAP-induced anti-inflammatory and antifibrogenic signaling results from enhanced expression of the Fc immunoreceptor FCGR2A/FcγRIIA and activation of an FCGR2A-mediated PTPN6/SHP-1 anti-inflammatory pathway, leading to increased engulfment of IgG into $LC3^{+}$ phagosomes. In patients with cirrhosis progressing to multi-organ failure (acute-on chronic liver failure), LAP is lost in monocytes, and can be restored by targeting FCGR2A-mediated PTPN6/SHP-1 signaling. These data suggest that sustaining LAP may open novel therapeutic perspectives for patients with end-stage liver disease.

Chronic liver injury exposes to liver fibrosis and potentially evolves to end-stage cirrhosis, cirrhosis decompensation and ultimately organ dysfunction and death. Cirrhosis lacks definitive treatment, and, as of today, liver transplantation remains the only option. A key feature of chronic liver injury is sustained hepatic and systemic inflammation, in particular originating from monocytes/macrophages, that drives the fibrogenic process and is crucial for progression to cirrhosis. Systemic inflammation also governs the development of multiorgan failure in patients with cirrhosis, a syndrome called acute-on chronic liver failure (ACLF), that exposes to a high risk of death and results from an acute burst in systemic inflammation over the chronic systemic inflammation already present in cirrhosis**.** Therefore, there is growing interest in the identification of the mechanisms that reprogram the monocyte/macrophage phenotype to control inflammation, in order to disrupt the detrimental sequence of events underlying progression of chronic liver diseases to cirrhosis and ACLF.

LC3-associated phagocytosis (LAP) is a phagocytic process that uses some but not all of the autophagy machinery to promote phagosome maturation and degradation of engulfed pathogens. In particular, LAP requires the class III phosphatidylinositol 3-kinase (PtdIns3K) complex, elements of the ubiquitination-like, protein conjugation system (ATG5, ATG7), and RUBCN (rubicon autophagy regulator), which is needed both at early stages of LAPosome formation and to stabilize and promote assembly of the phagocytic CYBB/NOX2 complex. Because LAP modulates the innate immune response, we recently investigated whether this process could be involved in the regulation of the inflammatory response during cirrhosis, and evaluated its impact on hepatic inflammation and fibrosis progression in human samples and animal models [1].

We observed that recruitment of LC3 to phagosomes (LAPosomes) is enhanced in

blood and liver monocytes from patients with early fibrosis or with end-stage cirrhosis, as well as in cells from mice with chronic liver injury, as compared to cells from healthy donors or control mice, respectively. Recruitment of LC3 to LAPosomes parallels that of key components of the LAP machinery, such as PIK3C3/Vps34 and the CYBA/p22phox subunit of the NADPH complex, but is not associated with activation of the early steps of macroautophagy. Investigation of the link between LAP activation and inflammation showed that the PtdIns3K inhibitor 3-methyladenine, the NADPH complex inhibitor DPI or a peptide disrupting the interaction between CYBA and RUBCN, exacerbate the inflammatory signature in monocytes from patients with cirrhosis. Mice deficient for LAP in myeloid cells, i.e. lacking RUBCN in the myeloid lineage, develop exaggerated hepatic inflammation and are more prone to develop liver fibrosis in response to chronic liver injury.

We next characterized the mechanisms underlying activation of LAP in cirrhotic monocytes and evaluated the expression of several plasma membrane LAP-triggering receptors. In macrophages, LAP is initiated by the engagement of various innate immune receptors, such as pattern-recognition receptors (i.e., toll like receptors, C-type lectin receptors, i.e. CLEC7A/Dectin-1), Ptd-Ser receptors (TIMD4/TIM4) or Fcγ receptors, in particular FCGR2A. We observed that gene and protein expression of *FCGR2A* is higher in monocytes from patients with cirrhosis as compared to healthy donors, whereas the expression of some other LAP-triggering receptors, including *CLEC7A* or *TIM4* is not modified. Interestingly, mice overexpressing human FCGR2A in myeloid cells show enhanced LAP in response to chronic liver injury, and are resistant to inflammation and liver fibrosis. In addition, overexpression of FCGR2A in monocytes from patients with cirrhosis is associated with a strong increase in IgG immunostaining in LAPosomes. Intriguingly, the serum from cirrhotic patients shows high amounts of uncomplexed IgG, as

compared to the serum of healthy donors. Therefore, during cirrhosis, IgG internalization by monocytes, most likely in an uncomplexed form, leads to the recruitment of the LAP machinery and to the initiation of an anti-inflammatory response (**Figure 1**).

An interesting point is that FCGR2A can trigger either pro- or anti-inflammatory signals, depending on the type of ligand. Thus, it is well known that crosslinking of FCGR2A by multimeric IgG immune complexes generates inflammatory signals. However, anti-inflammatory signals are also transmitted following FCGR2A interaction with uncomplexed IgG. This pathway involves an immunoreceptor tyrosine–based activation motif (ITAMi)-dependent signal, following recruitment and activation of the phosphatase PTPN6/SHP-1 to the receptor. We observed that the activated phosphorylated form of PTPN6/SHP-1 (p-PTPN6/SHP-1^{Y536}) colocalized with LAPosomes in monocytes from patients with cirrhosis. Silencing of PTPN6/SHP-1 blocks both the increase in LC3 expression and its interaction with FCGR2A in immunoprecipitates from monocytes transfected with the human FCGR2A. Interestingly, association of FCGR2A-PTPN6/SHP-1 with LC3 is also observed in FCGR2A immunoprecipitates from monocytes of patients with cirrhosis but not from healthy donors. Altogether, these data demonstrated that during cirrhosis, engulfment of IgG by monocytes promotes FCGR2A-mediated ITAMi signaling, which recruits LC3 and promotes LAP (**Figure 1**).

We finally evaluated the status of LAP in blood monocytes from patients with cirrhosis progressing to ACLF. We found that the increases in LAP and FCGR2A expression, observed in monocytes from patients with stable cirrhosis, is lost in monocytes from patients with ACLF, but that LAP can be restored by incubation of these cells with anti-FCGR2A $F(ab')_2$ fragments.

In conclusion, our data identify LAP as an anti-inflammatory pathway in blood and liver monocytes from patients and mice with chronic liver injury, that constrains both systemic and hepatic inflammation, with potent antifibrogenic effects and protection against evolution toward a more severe disease stage (**Figure 1**). They also provide a novel unsuspected link between LAP and PTPN6/SHP-1-inhibitory ITAMi signaling, that conveys the anti-inflammatory properties of FCGR2A (**Figure 1**). From a clinical point of view, these results open potential therapeutic perspectives of sustaining LAP to prevent progression to end-stage liver diseases. They also invite research to delineate whether further activation of LAP may constitute an efficient antifibrogenic strategy in the liver, and more generally in organs where inflammation-driven fibrosis is a common response to tissue injury such as kidney, heart or lung.

Reference

1. Wan J, Weiss E, Ben Mkaddem S et al, Sci Transl Med, 2020, 12, eaaw8523. doi: 10.1126/scitranslmed.aaw8523.

Figure 1. LAP is an anti-inflammatory and anti-fibrogenic mechanism in the liver, via a mechanism involving uncomplexed IgG-FCGR2A-PTPN6/SHP-1-ITAMi signaling. For simplicity, ATG stands for all ATG proteins recruited to the LAPosome membrane.

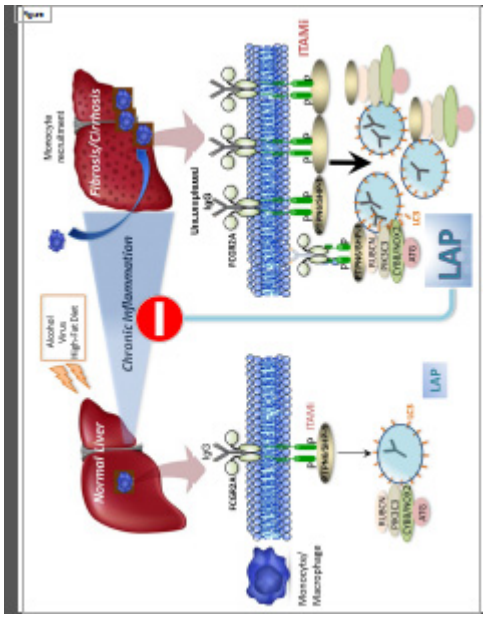

Fig 1