

# Hepatocyte-Derived Microparticles Promote NLRP3 Inflammasome Activation in HepG2 Cells

E. Chiazza<sup>1</sup>, E. Benetti<sup>1</sup>, M. Rogazzo<sup>1</sup>, S. Cannito<sup>2</sup>, C. Paternostro<sup>2</sup>, E. Novo<sup>2</sup>, M. Parola<sup>2</sup>, R. Fantozzi<sup>1</sup>, M. Collino<sup>1</sup>

<sup>1</sup>Dept. of Drug Science and Technology and <sup>2</sup>Dept. of Clinical and Biological Sciences, University of Turin, Italy

It has been recently shown that fat-laden hepatocytes, following exposure to saturated but not unsaturated free fatty acids, release microparticles (MPs) that may act as critical signals for the progression of liver injury in developing nonalcoholic steatohepatitis (NASH) (Smith BW et al., 2011; Povero D et al., 2012). However, so far the specific cellular and molecular pathways that mediate MPs-induced liver injury are not yet characterized. One of the most recently identified signaling pathway, whose activation affects many metabolic disorders, is the 'inflammasome', a multiprotein complex composed of NLRP3 (nucleotide-binding domain and leucine-rich repeat protein 3), ASC (apoptosis-associated speck-like protein containing a CARD) and procaspase-1. NLRP3 inflammasome activation leads to the processing and secretion of the proinflammatory cytokines interleukin (IL)-1 $\beta$  and IL-18 (Benetti et al., 2013, *in press*). Here we have investigated the role of MPs as a stimulus leading to inflammasome activation in human HepG2 cells, a well-known in vitro model system for the study of polarized human hepatocytes. The effects evoked by MPs were compared with those obtained with palmitic acid (PA) and lipopolysaccharide (LPS) treatment, both previously demonstrated to induce NLRP3 inflammasome activation in rodent hepatocytes. MPs were collected and purified following their release by fat-laden HepG2 (i.e., HepG2 exposed for 24 hr to 0.25 mM PA). HepG2 resting cells were then incubated, from 15 min up to 72 h, with MPs, LPS (100 ng/mL-1  $\mu$ g/mL) or PA (150 – 500  $\mu$ M). Expression of NLRP-3, pro-caspase and cleaved caspase 1, pro-IL-1 and cleaved IL-1 $\beta$  was evaluated by Western blot analysis in cell lysates, whereas ELISA assays were used to measure levels of IL-1 $\beta$  and IL-18 released in culture medium. All the three stimuli (MPs, LPS and PA) upregulated NLRP-3, pro-caspase 1 and pro-IL-1 $\beta$  expression in HepG2 cells and induced IL-1 $\beta$  released in the medium. Exposure of HepG2 cells to MPs also resulted in an increased release of IL-18. Maximal level of induction was detected at 1-3 hrs following LPS and was delayed at 16-48 h in cells exposed to PA or MPs. Overall, these results demonstrate that fat-laden hepatic cells, by releasing MPs, can efficiently trigger inflammasome activation, thus identifying a new molecular mechanisms of inflammation in NASH pathogenesis. The identification of selective pharmacological tools able to affect expression and/or activity of this novel pathway could hopefully lead to novel and specific therapies for the prevention and treatment of NASH.

Smith BW. et al., (2011) *Nat Rev Endocrinol.* 7, 456-65.

Povero D. et al., (2012) *Hepatology.* 56, 251A.

Benetti E. et al., (2013) *Mediators Inflamm.* *In press*