# The Unexpected Role of Neutrophils for Resolving Liver Inflammation by Transmitting MicroRNA-223 to Macrophages

Neutrophils are commonly recognized as prototypic inflammatory cells: They invade the liver upon injury, have a short half-life in inflamed tissue, and aggravate injury as well as inflammatory reactions. This has been conclusively demonstrated for acute acetaminophen injury in mice, where myeloid-cell–specific receptor for advanced glycation end products deficiency dramatically altered neutrophil recruitment to necrotic areas, and high-mobility group box 1 epithelial cell deficiency in the liver prevented all acetaminophen-induced mortality.[1] Similar findings in alcoholic hepatitis in humans support the detrimental roles of neutrophils in aggravating tissue injury and inflammation.[2] On the other hand, neutrophil recruitment impairment does not seem to influence bile duct ligation or acute or long-term carbon-tetrachloride–induced liver injury and fibrosis.[2]

The current study by Jimenez Calvente et al. challenged the "traditional view" on detrimental effects of neutrophils in liver disease by demonstrating that neutrophils mediate the resolution of liver inflammation and fibrosis through microRNA-223 (miR-223) delivery to liver macrophages.[3] The investigators used an *in vivo* model of neutrophil depletion by injecting Ly6G monoclonal antibodies during recovery phases of two different chronic liver disease models in mice, namely carbon tetrachloride repeated gavages or methionine and choline–deficient diet. In this setup, isotype-injected mice recovered from tissue injury, whereas neutrophil-depleted animals exhibited prolonged tissue damage, inflammation, and fibrosis. This work further described that neutrophil depletion led to a down-regulation of miR-223 levels in hepatic macrophages. Consequently, macrophages exhibited an increased NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome activation and a proinflammatory phenotype, as revealed by increased Ly6C staining, decreased CD14 and CD163 staining, and increased proinflammatory gene expression in neutrophil-depleted mice. The study elaborated on the cell-to-cell communication between neutrophils and macrophages, and based on a granulocyte membrane marker (CD177) staining and adoptive cell transfer experiments, the investigators suggested that neutrophil-derived extracellular vesicles (EVs) deliver miR-223 to liver macrophages, thus controlling their activation state and promoting inflammation resolution (Fig. 1).[3]

The work by Ariel Feldstein's group explores the exciting idea that neutrophils are not only to be considered as the harmful tissue-attacking immune cells, but also as crucial actors of tissue physiology restoration.[3] Interestingly enough, this is mediated by shaping the phenotype of hepatic macrophages. This work fits perfectly to another recent study by Yang et al. that identified the beneficial effects of neutrophil-derived reactive oxygen species (ROS) in polarizing macrophages toward an alternative or proregenerative and anti-inflammatory phenotype.[4] Thereby, neutrophil biology is much more elaborate than previously established, given that they are seemingly able to adopt a plethora of phenotypes tightly regulated by the integration of numerous signals derived from the microenvironment. Novel technologies like single-cell RNA sequencing or multispectral imaging currently allow a much more detailed insight into the different cell subpopulations present in the liver, for instance highlighting the functional diversity of macrophages during chronic liver injury.[5] Not so much is known about the heterogeneity of neutrophils, and in light of the recent aforementioned findings, advances are awaited in the near future to answer these questions. It must be kept in mind, however, that the model herein used to deplete neutrophils may not be optimal. The use of anti-Ly6G antibodies induces neutrophil death *in situ*; thus, the accumulation of cell debris in the injured tissue may exacerbate immune

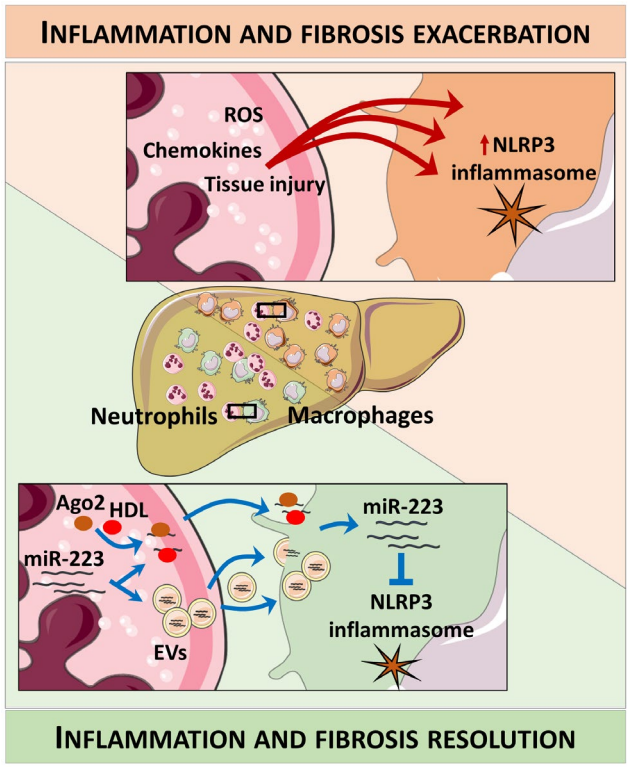


**FIG. 1.** Neutrophils participate in liver inflammation resolution by miR-223 delivery to macrophages. Jimenez Calvente et al. showed that neutrophils are crucial for tissue inflammation resolution in liver disease models, through transferring miR-223 to macrophages by EVs, or possibly also in the non-membrane-bound form associated with high-density lipoprotein (HDL) or Ago2. This study further proposed that miR-223 represses Nlrp3 at the transcriptional level, thus inhibiting proinflammatory gene expression and favoring macrophage polarization toward a regenerative phenotype. This is opposed to the classical view that neutrophils would essentially participate in liver injury and inflammation aggravation.

cell (i.e., macrophage) activation and phenotype, thus promoting liver fibrosis. Thorough characterization of immune cell phenotypes must take into account such potential drawbacks.

The data presented by Jimenez Calvente et al. indicate that neutrophils could be a source of miR-223 that is transmitted by microvesicles to shape macrophage phenotypes. However, this may possibly represent only a part of the full picture, because previous studies have claimed that monocytes and macrophages represent a potent source of miR-223, and even epithelial cells from the liver (e.g., hepatocytes) might express miR-223, or be the targets of immune-cell–derived miR-223 as well.[6] Moreover, miR-223 could also be transferred in a non-membrane-bound form associated with lipoproteins or Argonaut 2 (Ago2; Fig. 1). Thorough characterization of the source and delivery mechanisms of miR-223, together with in-depth analyses of intercellular communication, may unravel more complex roles of miR-223 in inflammatory diseases and highlight potential therapeutic applications.

Interestingly, miR-223 was recently implicated in the pathogenesis of nonalcoholic steatohepatitis (NASH) and hepatocarcinogenesis. miR-223 was found to be increased in mice with experimental steatohepatitis as well as in human NASH samples, whereas miR-223-deficient mice developed more severe steatohepatitis and liver cancer upon high-fat-diet feeding, suggesting that miR-223 represents a key counter-regulatory pathway for limiting disease progression.[6] In fact, miR-223 targeted inflammatory and tumorigenic genes in hepatocytes.[6] Similarly, miR-223 down-regulation led to increased intestinal inflammation, attributed to more potent macrophage activation.[7]

Collectively, these experimental studies demonstrate that the endogenous microvesicular delivery of miR-223 seems to have beneficial effects on macrophages during injury resolution,[3] on hepatocytes during NASH progression,[6] as well as on limiting intestinal inflammation.[7] The fundamental relevance of this pathway makes it tempting to speculate that exogenous delivery of miR-223 and/or augmenting the beneficial roles of neutrophils during tissue regeneration might be interesting therapeutic approaches in chronic liver diseases, particularly NASH and fibrosis.

The current work also broadens our view on neutrophils—instead of viewing them as passive responders to tissue damage, neutrophils are active orchestrators of inflammation and repair mechanisms in the liver (Fig. 1). Nonetheless, shaping macrophages toward "repair macrophages" is only one aspect of neutrophil biology in the liver. Additional mechanisms include phagocytosis, release of ROS, bactericidal activity, and formation of neutrophil extracellular traps; all of which have been related to exacerbation of inflammation, autoimmunity, and tumor progression.[8] Our increasing insight into immune cell heterogeneity and molecular mechanisms of immune cell functionality

underline once more the need for immune-modulatory, rather than immunosuppressive, therapeutic approaches to counteract inflammatory, fibrogenic, or carcinogenic pathways driving chronic liver diseases.

**Adrien Guillot, Ph.D.**
**Frank Tacke, M.D., Ph.D.**
*Department of Hepatology & Gastroenterology*
*Charité Universitätsmedizin Berlin*
*Berlin, Germany*

## REFERENCES

1) **Huebener P**, **Pradere JP**, **Hernandez C**, Gwak GY, Caviglia JM, Mu X, et al. The HMGB1/RAGE axis triggers neutrophil-mediated injury amplification following necrosis. J Clin Invest 2015;125:539-550.
2) Moles A, Murphy L, Wilson CL, Chakraborty JB, Fox C, Park EJ, et al. A TLR2/S100A9/CXCL-2 signaling network is necessary for neutrophil recruitment in acute and chronic liver injury in the mouse. J Hepatol 2014;60:782-791.
3) **Jimenez Calvente C**, **Tameda M**, Johnson CD, Del Pilar H, Chin Lin Y, Andronikou N, et al. Neutrophils contribute to spontaneous resolution of liver inflammation and fibrosis via microRNA-223. J Clin Invest 2019;130:122258.
4) **Yang W**, **Tao Y**, Wu Y, Zhao X, Ye W, Zhao D, et al. Neutrophils promote the development of reparative macrophages mediated by ROS to orchestrate liver repair. Nat Commun 2019;10:1076.
5) Krenkel O, Hundertmark J, Abdallah AT, Kohlhepp M, Puengel T, Roth T, et al. Myeloid cells in liver and bone marrow acquire a functionally distinct inflammatory phenotype during obesity-related steatohepatitis. Gut 2019 May 10. pii: gutjnl-2019-318382. https://doi.org/10.1136/gutjnl-2019-318382. [Epub ahead of print]
6) He Y, Hwang S, Cai Y, Kim SJ, Xu M, Yang D, et al. MicroRNA-223 ameliorates nonalcoholic steatohepatitis and cancer by targeting multiple inflammatory and oncogenic genes in hepatocytes. Hepatology 2019 Apr 9. https://doi.org/10.1002/hep.30645. [Epub ahead of print]
7) Neudecker V, Haneklaus M, Jensen O, Khailova L, Masterson JC, Tye H, et al. Myeloid-derived miR-223 regulates intestinal inflammation via repression of the NLRP3 inflammasome. J Exp Med 2017;214:1737-1752.
8) Honda M, Kubes P. Neutrophils and neutrophil extracellular traps in the liver and gastrointestinal system. Nat Rev Gastroenterol Hepatol 2018;15:206-221.

Author names in bold designate shared co-first authorship.

*A.G. is a recipient of a Humboldt Research Fellowship for Postdoctoral Researchers. This work has been supported by the German Research Foundation (DFG CRC1382).*



*View this article online at wileyonlinelibrary.com.*

*DOI 10.1002/hep.30925*

*Potential conflict of interest: Dr. Tacke consults for, advises for, is on the speakers' bureau for, and received grants from Inventiva, Allergan, and Bristol-Myers Squibb. He consults for, advises for, and is on the speakers' bureau for Bayer, GalMed, Gilead, AbbVie, and MSD. He received grants from Galapagos.*

# Biliary Atresia: Biliary-Enteric Drainage or Primary Liver Transplant?

The article from LeeVan et al.,[1] entitled "Biliary-enteric drainage vs primary liver transplant as initial treatment for children with biliary atresia," is a retrospective cohort study that examines long-term outcomes for children with biliary atresia (BA) through analysis of information obtained from the California Office of Statewide Health Planning and Development (OSHPD), an administrative database. Specifically, the researchers compare outcomes between a cohort of BA patients who underwent a biliary enteric drainage (BED) procedure (presumably a Kasai hepatoportoenterostomy) as the primary therapy to those who underwent a primary liver transplant (pLT) without a previous BED. This study is particularly interesting, because despite our best efforts, adjuvant therapy to BED has not proven successful in improving transplant-free outcomes. Two recent studies have shown adjuvant corticosteroids or intravenous immunoglobulin to be ineffective in improving short-term outcomes.[2,3]

The results of the current study show that overall survival in the BED group was inferior to the pLT group at 5-, 10-, 15-, and 20-year time points. They also demonstrate that a significant contributor to the inferior outcomes is the relatively poor outcomes of salvage liver transplant following BED compared to pLT. Finally, they show that while the difference in outcomes between BED and pLT were not significantly different before 2002, they are significant after 2002 largely attributable to a significant improvement in pLT outcomes. Ultimately, the researchers are attempting to answer the question of whether or not BA patients should ever undergo a BED or whether they would be better off going straight to liver transplantation. While this is an appropriate question to ask given the significant improvement in overall pediatric liver transplantation outcomes, this study does