White blood cells play unexpected role in clearing out dead liver cells

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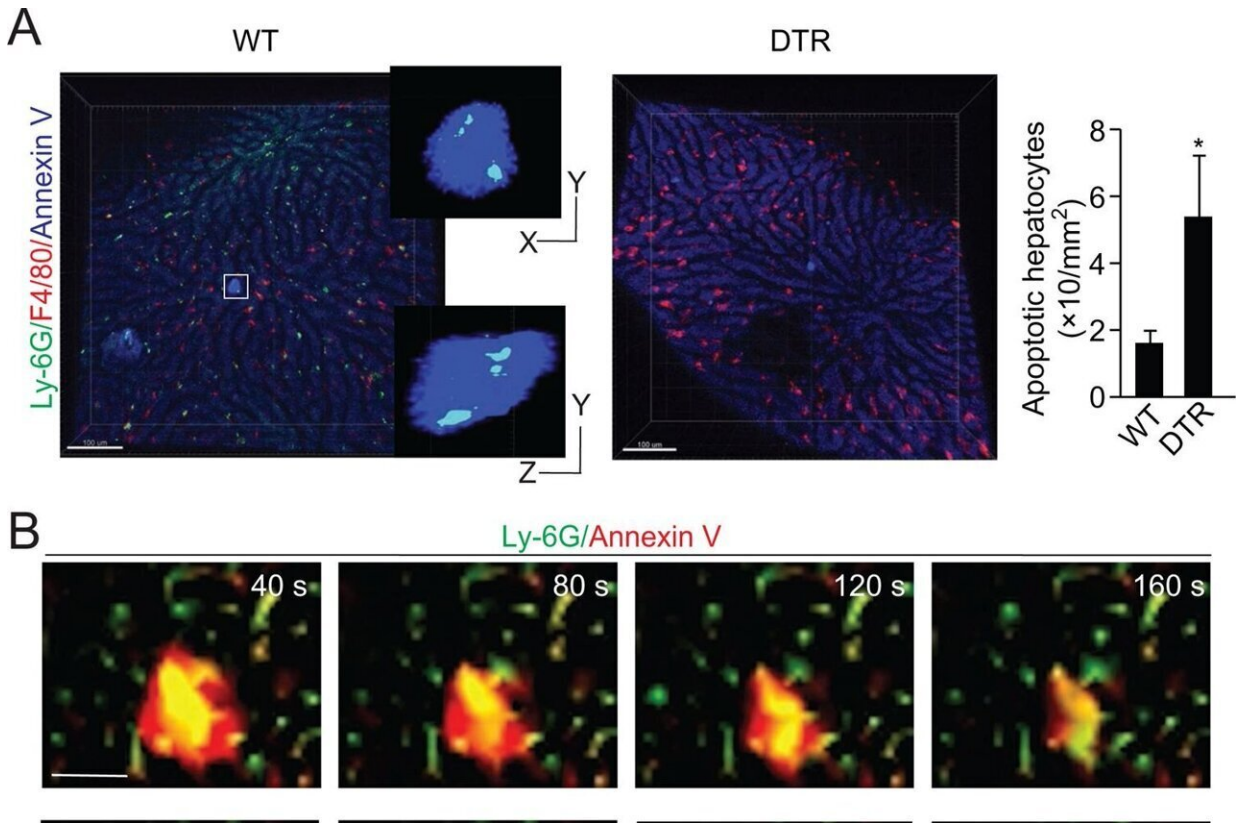

Neutrophil burrowing into apoptotic hepatocytes. (A) Intravital microscopy images of liver tissues from WT and MRP8cre/DTR mice. Neutrophils are labeled with an i.v. injection of anti-Ly6G antibody (green), KCs are labeled with anti-F4/80 antibody (red), and apoptotic cells are labeled with Annexin V (blue). Neutrophils inside and KCs associated with apoptotic hepatocytes are detected and analyzed with IMARIS software as described in Methods. The distances from neutrophils to the apoptotic hepatocyte border are recorded in table S6. A total of 24 apoptotic cells were observed in the WT liver with an

average of 2 burrowed neutrophils. Scale bar, 100 μm. (B) Intravital image sequences of neutrophils phagocytosing apoptotic hepatocytes in mouse livers at indicated time points. Neutrophils are labeled with an i.v. injection of anti-Ly6G antibody (green), and apoptotic cells are labeled with Annexin V (red). A total of 13 apoptotic cells with burrowed neutrophils were observed in 12 WT mouse livers. Scale bar, 20 μm. (C) Electron microscopy images of apoptotic mouse hepatocytes occupied by neutrophils. The apoptotic hepatic nucleus (AN) is evident by distorted nuclear membrane (pointed by black arrowheads). The neutrophils are indicated by white arrowheads with a characteristic multilobed nucleus. 29 apoptotic cells with burrowed neutrophils were observed. Scale bar, 5 μm. Data are representative of three independent experiments. Credit: (2023). DOI: 10.7554/eLife.86591.1

A type of white blood cell usually associated with immune responses to foreign particles may have another role in clearing out liver cells that have undergone apoptosis—where cells are programmed to die in a controlled manner.

The study, published today as a Reviewed Preprint in *eLife*, provides what the editors describe as solid evidence that neutrophils destroy liver cells going through apoptosis by burrowing into them—a process the authors have called 'perforocytosis'. The findings also suggest that a lack of neutrophils may be a cause of human autoimmune liver disease (AIL), with potential implications for new therapeutic strategies against the disease.

Billions of apoptotic cells are removed daily in adults by a group of immune cells called phagocytes. Neutrophils represent around 50–70% of the total white blood cell population in humans and are a type of phagocyte. However, unlike other phagocytes, they were widely assumed to be excluded from apoptotic cells, as they promote inflammation which could damage nearby healthy cells and tissues. The current

findings now challenge that assumption.

"Although apoptotic cells are well characterized, they are not often found within human samples, possibly because they are removed so efficiently by phagocytes," says co-lead author Luyang Cao, Associate Investigator in the Department of Neurosurgery, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, China. "This means that the specific phagocytes responsible for the removal of apoptotic cells remain unknown, and we do not know if they are specific to different tissues in the body."

To identify the phagocytes responsible for removing apoptotic cells in the liver, the team obtained cells from the liver tissue of patients with tumors caused by hepatocellular carcinoma or hepatic hemangioma. They used two different staining techniques to confirm which cells in the sample were apoptotic.

In a total of 281 apoptotic liver cells from the livers of 32 patients, the team noticed that each cell was engorged by the presence of up to 22 neutrophils. It has previously been suggested that a type of phagocyte called Kupffer cells were responsible for the clearance of apoptotic liver cells, but when the researchers searched for Kupffer cells in the samples, they found that very few were present.

They therefore hypothesized that neutrophils were the primary phagocyte for the removal of dead liver cells through the process they called perforocytosis. This contrasts to the usual process of engulfing apoptotic cells that most other phagocytes use.

To confirm the mechanism by which neutrophils remove apoptotic liver cells, the team sought to visualize the process in mouse livers using intravital microscopy—a live imaging technique that allows biological processes to be viewed in real time within living organisms. They labeled

liver cells with a protein called Annexin V and neutrophils with an anti-Ly6G antibody.

Consistent with their findings in human samples, the team observed that neutrophils burrowed into and cleared dead liver cells in the mice. The process was fast and rigorous, with the dead cells completely digested in four to seven minutes.

"Our discovery of neutrophils burrowing into and clearing out apoptotic liver cells helps to solve some of the mysteries surrounding the apoptotic clearance process," says co-corresponding author Hexige Saiyin, Assistant Professor in the State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, China.

Next, the team sought to investigate whether reducing the neutrophil population in mice impacts the clearance of apoptotic liver cells. In a sample of cells from the livers of neutrophil-depleted mice, the percentage of apoptotic cells was significantly higher than in normal mice—0.92% and 0.2%, respectively—suggesting that neutrophil depletion impairs the clearance of apoptotic cells. They also noticed the presence of other phagocytes in the neutrophil-depleted mice, implying a compensatory role of other phagocytes in the absence of neutrophils.

The defective clearance of apoptotic cells is often linked with autoimmune diseases, such as AIL. In the neutrophil-depleted mice, the team noticed an increase in autoantibodies—immune cells that mistakenly attack the body's own healthy cells instead of foreign bodies such as viruses or bacteria.

This increase was unaffected by antibiotic treatments and present only in neutrophil-depleted mice, not in mice with other phagocyte depletions. This implies that neutrophil depletion is associated with impaired apoptotic liver cell clearance and, subsequently, the generation of

autoantibodies that may lead to AIL disease. The team consolidated this finding by analyzing biopsy samples from human patients with AIL disease. Once again they found that, in each patient, the neutrophil-mediated clearance of apoptotic cells was impaired.

The authors say that more research is needed to better understand the process and significance of perforocytosis, as well as whether perforocytosis occurs in other organs besides livers. The next important step is how to apply this newly identified apoptotic clearance mechanism to the clinical treatment of AIL.

"Since the failure to clear dead cells is linked to inflammatory and autoimmune diseases, further insights into the critical role that neutrophils play in apoptotic clearance may have important implications for the treatment of these diseases. We recently have screened and identified several compounds which markedly enhanced neutrophil perforocytosis and demonstrated great therapeutic values to cure AIL in mouse models," concludes senior author Jingsong Xu.

More information: Luyang Cao et al, An Unexpected Role of Neutrophils in Clearing Apoptotic Hepatocytes In Vivo, *eLife* (2023). DOI: 10.7554/eLife.86591.1

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