An Unexpected Line of Defense: Hepatoprotective Eosinophils in Ischemia-Reperfusion Injury

Liver transplantation is a highly successful treatment for patients with acute liver failure, end-stage liver disease, and primary hepatic malignancies. However, due to organ shortage, early allograft failure and a concomitant rise in chronic liver diseases, the number of patients requiring liver transplantation is disproportionately higher than organs successfully transplanted. To meet the growing demand, extended criteria donor organs (e.g., older, steatotic, prolonged ischemia time) were implemented. These "marginal" organs are highly susceptible to ischemia-reperfusion injury (IRI), resulting in poor graft function and survival in affected patients. Therapeutic or preventive modalities for IRI are limited, and our understanding of the pathogenesis is incomplete. IRI generally occurs after temporary deprivation and restoration of blood supply, resulting in ischemic organ damage and inflammation-mediated reperfusion injury. On a cellular level, ischemia causes oxidative stress, resulting in hepatocyte death and Kupffer cell activation.[1] Reperfusion causes sterile inflammation, infiltration of neutrophils, and release of pro-inflammatory cytokines. Immune regulation containing IRI still remains poorly understood. However, IRI not only occurs in iatrogenic settings such as transplantation, but also after traumatic injury, when immune regulatory mechanisms might have developed. Thus, a deeper understanding could provide novel therapeutic strategies. A recent intriguing study by Ju et al. expanded our knowledge on regulatory mechanisms in IRI and identified an unexpected source of liver protection: Eosinophils rapidly invaded liver allografts of 22 transplant recipients in the first 2-3 hours.[2] The authors recapitulated this observation in a mouse model of hepatic IRI, noting a stark increase in liver eosinophils over the first 24 hours after IRI (Fig. 1). Surprisingly, inhibiting eosinophils using antibody-mediated depletion or mice deficient in eosinophils, aggravated hepatic IRI, and increased liver necrosis and inflammatory cytokines. Mechanistically, invading eosinophils expressed high levels of the receptor suppression of tumorgenicity 2 (ST2), and ST2 worked in conjunction with its ligand IL-33 to mediate protection. Both ST2-deficient and IL-33-deficient mice failed to recruit eosinophils after IRI and consequently exhibited a higher degree of liver damage. Eosinophils are known to produce the Th2 cytokines IL-4 and IL-13. In expanding the mechanistic details, the authors specifically depleted either IL-4 or IL-13 in eosinophils using IL-4/IL-$13^{fl/fl}$ x $eoCre^{+/x}$ mice, which were subjected to IRI. Although blocking IL-4 had no effect, specifically ablating IL-13 in eosinophils increased liver necrosis and thus failed to provide eosinophil-mediated protection. Finally, the authors noted a reciprocal relationship between eosinophils and neutrophils. Mice deficient in eosinophils had greatly increased numbers of neutrophils and increased liver damage, suggesting a regulatory role for eosinophils by containing unhinged neutrophil recruitment.

The therapeutic potential of eosinophils, the ST-2/IL-33 axis, and subsequent release of IL-13 was further investigated. In a set of adoptive transfer experiments, eosinophil-mediated protection was transferable by pre-emptively administering bone marrow–derived eosinophils to eosinophil-deficient mice. Additional protection was also achieved in wild-type mice after transfer of bone marrow–derived eosinophils before IRI. Interestingly, in IL-33 deficient mice, only IL-33-stimulated eosinophils dampened liver injury, indicating IL-33 was required to initiate eosinophil-mediated protection. Finally, recombinant IL-13 given therapeutically reduced IRI in mice. In summary, this study identified a hitherto unknown role for eosinophils as regulatory cells, producing IL-13 through the ST-2/IL-33 axis to attenuate liver injury (Fig. 1). Importantly, the authors demonstrated a beneficial effect by adoptively transferring eosinophils or by administering IL-13 in preclinical models of IRI, laying the groundwork for future translational studies aimed at improving transplant-induced IRI.

Eosinophils are leukocytes typically known as cytotoxic effector cells in allergic inflammation and

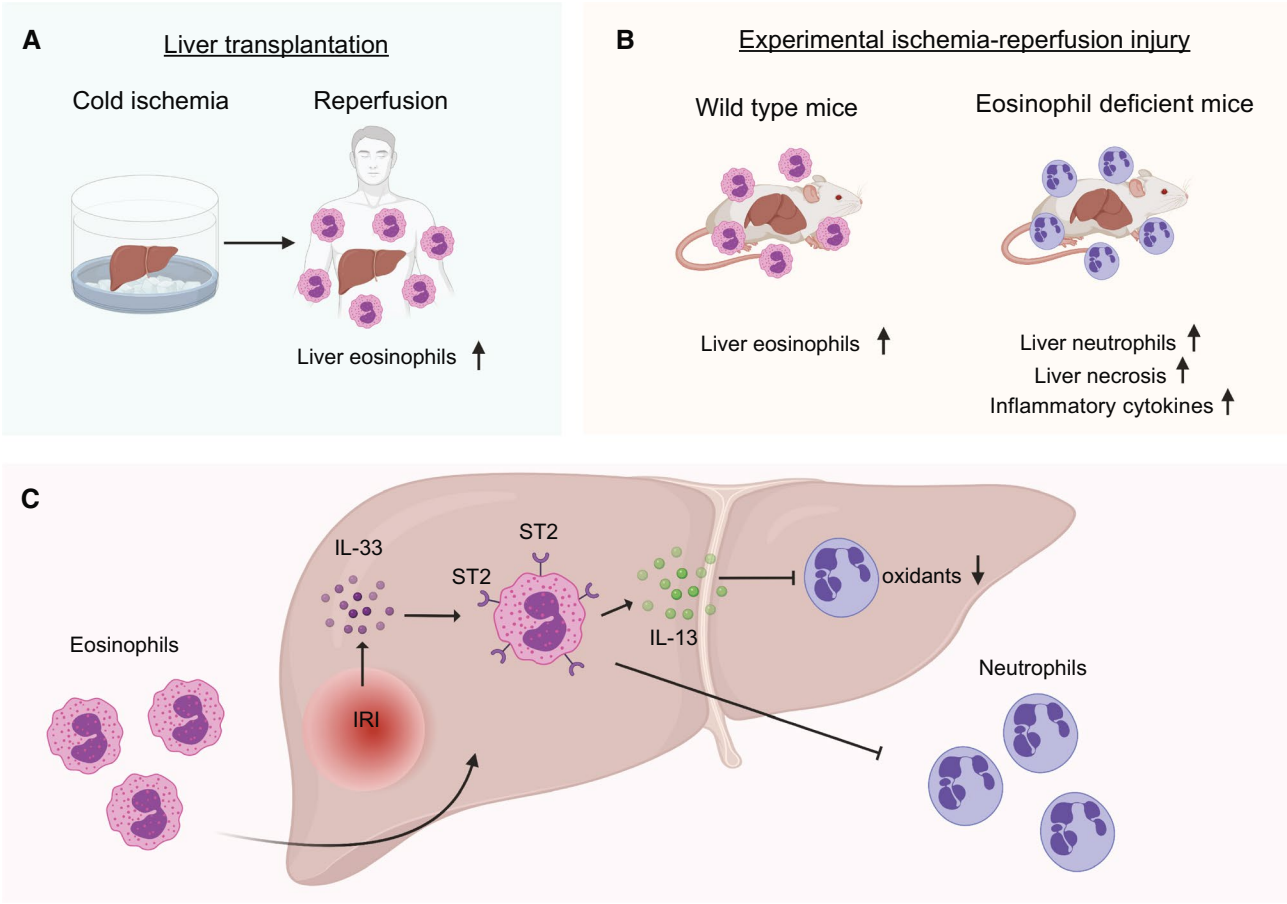

FIG. 1. Eosinophils accumulate in IRI and attenuate liver damage. Eosinophils rapidly increase in liver allografts (A) and in mice subjected to experimental IRI (B). Mice deficient in eosinophils showed increased neutrophils and liver injury. (C) IL-33 released during injury signals via ST2 on eosinophils to induce IL-13 liberation, in turn inhibiting neutrophil recruitment and neutrophil-derived oxidants.

parasitic infection. Eosinophils promote inflammation through secretion of cytokines and release of toxic granules, thereby stimulating immune cell trafficking and function.[3] However, the 'traditional view' of eosinophils as destructive effector cells is evolving, and more recent studies demonstrate various regulatory properties by promoting injury resolution through release of anti-inflammatory cytokines or direct inhibition of effector cells.[4] With regard to liver injury and repair, eosinophils were shown to stimulate hepatocyte proliferation after toxic injury and partial hepatectomy by producing IL-4 and signaling through the IL-4 receptor on hepatocytes.[5] Along those lines, the study by Ju et al. now identifies eosinophils as hepatoprotective cells in liver IRI and emphasizes the immune-regulatory effects of eosinophil-released IL-13.[2] Whether eosinophil-derived IL-13 also contributes to liver regeneration after IRI by directly stimulating hepatocyte proliferation remains unknown.

This serendipitous observation is intriguing and offers therapeutic potential in transplant-induced IRI; however, a few issues need to be considered. First, eosinophils in the livers of transplant recipients were assessed during cold ischemia (before IRI) and after transplantation (following IRI), but not compared against baseline and thus might have been artificially decreased by cold preservation. Second, while eosinophils and neutrophils showed reciprocal recruitment kinetics in mice, the precise mechanism by which eosinophils or eosinophil-derived IL-13 inhibited neutrophil recruitment remains obscure. Of note, an inhibiting role of IL-13 on neutrophil recruitment was previously described in hepatic *Schistosomiasis*.[6] Furthermore, in allergic inflammation, where Th2-type cytokines such as IL-4 and IL-13

predominate, neutrophils are conspicuously absent. A number of recent studies have uncovered a reciprocal regulation of type II immune responses and neutrophil recruitment, identifying a checkpoint pathway to prevent excessive tissue injury.[7] Accordingly, neutrophil egress and chemotaxis was directly curtailed by IL-4/IL-13 signaling through type I and type II IL-4 receptors on neutrophils.[7] This compelling regulatory loop might explain the findings in hepatic IRI, but more evidence is necessary to understand the balance between neutrophils and eosinophils. Furthermore, the authors quantified liver injury only during the first 24 hours after IRI. While often associated with tissue damage, the view of neutrophils as unrestrained cytotoxic cells is changing, and they are emerging as cells with important repair properties.[8] In various liver injury models, it was shown that early neutrophil depletion was accompanied by smaller injury size yet translated into defective healing after a few days.[8] Thus, it is worthwhile investigating whether reduced neutrophil recruitment early has inauspicious effects at later stages. An additional point worth raising is that the mouse model of IRI only partially mimics IRI in liver transplantation, and by temporarily clamping blood flow, causes retrograde blood stasis in the intestine, leading to barrier disruption and leaky gut. Ju et al. found IL-33 as the main cytokine to induce the eosinophil-driven protection, yet the source of IL-33 was unknown. IL-33 is an "alarmin" that is released by different cells such as endothelial and epithelial cells in a variety of tissue perturbations; thus, an additional source of IL-33 in hepatic IRI could be the perturbed intestine. IL-33 mediates an array of immune modulatory properties, primarily through ST2 on regulatory immune cells, such as regulatory T cells and innate-like lymphocytes 2 (ILC2s).[2] Although the authors demonstrated that eosinophils were the most abundant ST2-expressing cells in the liver, regulatory T cells and ILC2s might still account for some of the protective effect, either directly in the liver or systemically through IL-33.

Hepatic IRI is an important iatrogenic sequela, and most experimental studies have focused on the ensuing effector cells causing hepatocellular damage. However, rather than diminishing the effector arm, balance might be therapeutically restored by strengthening regulatory immunity. As such, the study by Ju et al. provides pioneering evidence for an overlooked cell type: protective eosinophils that rapidly invade the liver, skewing the microenvironment toward repair by releasing IL-13 and hopefully spawning future studies investigating the therapeutic potential of eosinophils.

Acknowledgment: Figure 1 was created with BioRender.com.

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Supported by the German Research Foundation (SFB/TRR296, CRC1382, Ta434/3-1, and Ta434/5-1) and the Berlin Institute of Health (BIH-Charité Clinician Scientist Program) funded by the Charité–Universitätsmedizin Berlin and the Berlin Institute of Health.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.31895

Potential conflict of interest: Nothing to report.