The Role of Myeloid Populations during Perinatal Liver Injury and Repair

Anas Alkhani¹,², Sarah Mohamedaly¹,², Amar Nijagal¹,²,*
¹Department of Surgery, University of California, San Francisco, CA, USA
²Liver Center, University of California, San Francisco, CA, USA

Abstract

Perinatal liver inflammation can have life-threatening consequences, particularly in infants and young children. An example of a hepatic inflammatory disease during infancy is biliary atresia (BA), an obliterative cholangiopathy that rapidly progresses to hepatic fibrosis and liver failure. The aggressive nature of BA in neonates compared to the pathogenesis of inflammatory liver diseases in adults, suggests that the mechanisms responsible for restoring tissue homeostasis following inflammation are impaired in affected infants. This article reviews our recent findings demonstrating that the relative abundance of Ly6cLo non-classical monocytes promotes resolution of perinatal liver injury in a murine model of perinatal hepatic inflammation. Our research also identifies a potential co-regulatory role between neutrophils and non-classical monocytes. Further work is needed to understand how neutrophils regulate other myeloid populations during perinatal liver inflammation. Elucidating the mechanisms that govern perinatal liver injury and repair may lead to the development of immune-directed therapies that can be used to mitigate the devastating effects of diseases like BA.

Keywords

Perinatal liver inflammation; Extramedullary hematopoiesis; Biliary atresia

Introduction

The maturation of the immune system is a complex process that undergoes major transitions during fetal and neonatal development [1]. Throughout this developmental window, the response to liver injury is dependent on the nature and timing of the insult [2]. For example, fetal damage following maternal infection can lead to the development of innate immune memory in the fetus, a process that may mitigate the impact of future insults [3]. It remains unknown how immune responses to injury are orchestrated and sustained during this period of hematopoietic development and immune system maturation.
In our recent study, “Ly6c<sup>Lo</sup> non-classical monocytes promote resolution of rhesus rotavirus-mediated perinatal hepatic inflammation,” we demonstrate the importance of monocyte subsets in mitigating inflammatory insults to the perinatal liver. Using a rhesus rotavirus (RRV) model of perinatal liver injury, we investigated changes in composition of the hepatic myeloid immune compartment in late-gestation fetuses and in neonatal pups [4]. Our initial experiments demonstrate that the late-gestation fetus is resistant to liver inflammation, while neonatal pups develop severe liver disease. We attributed the resistance to inflammation in the late-gestation fetus to the physiological abundance of Ly6c<sup>Lo</sup> non-classical monocytes observed at this time. Since Ly6c<sup>Lo</sup> non-classical monocytes exert anti-inflammatory and pro-reparative functions [5–7], we hypothesized that the abundance of these cells plays a role in rendering the fetus resistant to RRV-mediated liver injury.

To test this hypothesis, we manipulated the myeloid compartment within the neonatal liver to resemble the fetal environment, thereby establishing an abundance of Ly6c<sup>Lo</sup> non-classical monocytes. Using anti-Ly6g targeted neutrophil depletion [8], and anti-CCR2 targeted Ly6c<sup>Hi</sup> classical monocyte depletion [9], we expanded the number of Ly6c<sup>Lo</sup> non-classical monocytes within RRV-injected neonatal livers. Importantly, antibody-mediated depletion of neutrophils and Ly6c<sup>Hi</sup> classical monocytes increased both the percentage and the absolute number of Ly6c<sup>Lo</sup> non-classical monocytes, indicating both a proportional and numerical expansion of these cells. Furthermore, the rise in Ly6c<sup>Lo</sup> monocytes was associated with disease resolution in the neonate, supporting the idea that Ly6c<sup>Lo</sup> non-classical monocytes play a role in resistance to perinatal liver injury and resolution of disease. To confirm Ly6c<sup>Lo</sup> non-classical monocytes are responsible for disease resolution and resistance [4], we used a Cx3cr1 small molecule inhibitor (AZD8797) to selectively block Ly6c<sup>Lo</sup> non-classical monocyte function in the setting of neutrophil and classical monocyte depletion [10–12]. Blockade using this small molecule inhibitor restored susceptibility to RRV-mediated injury, supporting the idea that Ly6c<sup>Lo</sup> non-classical monocytes mitigate perinatal liver inflammation [4].

**Neutrophil-mediated Regulation of Inflammation and Tissue Repair**

Recent evidence suggests that neutrophils play a vital role in regulating and orchestrating inflammation and tissue repair through their interaction with the innate and adaptive immune systems [13–16]. Several studies have suggested that neutrophil absence may result in chronic injury and sequelae by altering the homeostatic response and the recruitment of monocytes in the setting of underlying tissue injury [13,16–23]. A study published by Horckmans et al. indicates that neutrophils play an essential role in regulating outcomes after cardiac tissue injury by altering the polarization state of macrophages [16]. The authors also demonstrate that the release of neutrophil gelatinase-associated lipocalin results in enhanced macrophage pro-reparative function at the injury site following myocardial infarction. Neutrophils were also found to promote tissue repair and regeneration via secretion of oncostatin M, which regulates macrophage accumulation at the site of cardiac tissue injury [24]. In the liver, neutrophils also induce cellular and necrotic debris clearance and promote vascular repair and growth after inflammation [25–28]. Furthermore, their absence in the setting of liver inflammation and cholestatic injury was found to exacerbate the liver injury and fibrosis, which in turn suggests a vital role for neutrophils in modulating...
the reparative mechanism within the liver after injury [29–31]. In RRV-injected and neutrophil-depleted pups, we observed an expansion of Ly6c\textsuperscript{Lo} non-classical monocytes [4], suggesting that the inverse correlation between neutrophils and Ly6c\textsuperscript{Lo} non-classical monocytes reveals a co-regulatory relationship between these two cell populations that are important for tissue repair and healing [14].

**The Liver and Spleen Actively Participate in the Kinetics of Hematopoiesis within the Neonate**

An intriguing mechanism by which neutrophils may influence other myeloid populations during inflammation may involve the regulation of myeloid precursors and hematopoietic progenitors [15]. We have observed changes to myeloid precursor populations after depletion of neutrophils during RRV-mediated inflammation (unpublished observations), prompting us to examine the role of emergency myelopoiesis role during perinatal liver injury.

The liver is known to serve as the primary hematopoietic organ throughout fetal life as it harbors the hematopoietic stem cell (HSC) niche [32]. Soon after birth in the mouse, the liver no longer serves as the primary site of hematopoiesis and its hematopoietic function is replaced by the BM, the latter establishing its permanent niche through adulthood [32–34]. However, the liver maintains relatively low levels of erythropoiesis and myelopoiesis, and retains a small population of HSCs and immune progenitors throughout adulthood [34,35]. Although the liver’s contribution to extramedullary hematopoiesis (EMH) in the setting of perinatal liver injury is not known, the liver can re-emerge as a site of EMH during immune and inflammatory damage and by doing so, contribute to the overall innate immune response [35,36].

In addition to the liver, the spleen can also contribute to the overall hematopoietic activity during neonatal development and inflammation via EMH [35,36]. However, the spleen’s preferential lineage outcome and the extent of its involvement in emergency hematopoiesis has not been determined. Splenic EMH differs within the first two weeks of life when compared to adulthood [36]. Wolber et al. have found that colonies of erythro-myeloid lineages, including myeloid forming colonies, erythroid forming colonies, and mixed erythroid/myeloid forming colonies, were detectable at large numbers in the spleen in the first two weeks of neonatal life. By adulthood, the spleen becomes more restricted to an erythroid lineage, although it still maintains minimal myeloid activity [36]. The spleen was also found to harbor myeloid progenitors that serve as precursors to Cd11b\textsuperscript{Hi} myeloid cells, and Ly6c\textsuperscript{Lo} and Ly6c\textsuperscript{Hi} monocytes in the setting of tissue injury and systemic disease [37,38]. These data support the idea that, in addition to the liver, the spleen may exhibit age-dependent hematopoietic activity that may govern immune responses during perinatal liver inflammation.

**Conclusion**

Understanding the immune response to liver injury during perinatal development is particularly important as the dynamic transitions in hematopoiesis during this time may
establish a unique environment that is susceptible to injury and inflammation. Since the liver and spleen are believed to contribute to hematopoiesis in the setting of perinatal liver inflammation, it is important to understand the kinetics of neonatal emergency hematopoiesis to highlight the possible mechanisms by which myeloid populations may contribute to liver injury and repair. Insights gained into perinatal liver inflammation mechanisms will undoubtedly lead to tailored therapies for infants and children who suffer from the devastating consequences of perinatal liver injury.

**References**

1. Johns JL, Christopher MM. Extramedullary Hematopoiesis: A New Look at the Underlying Stem Cell Niche, Theories of Development, and Occurrence in Animals. Vet Pathol. 2012 May 1;49(3):508–23. [PubMed: 22262354]

2. Apostol AC, Jensen KDC, Beaudin AE. Training the Fetal Immune System Through Maternal Inflammation—A Layered Hygiene Hypothesis. Front Immunol [Internet]. 2020 [cited 2020 Sep 12];11. Available from: 10.3389/fimmu.2020.00123/full

3. Mitroulis I, Rupova K, Wang B, Chen L-S, Grzybek M, Grinenko T, et al. Modulation of Myelopoiesis Progenitors Is an Integral Component of Trained Immunity. Cell. 2018 Jan 11;172(1–2):147–161.e12. [PubMed: 29328910]

4. Alkhani A, Levy CS, Tsui M, Rosenberg KA, Polovina K, Mattis AN, et al. Ly6c Lo non-classical monocytes promote resolution of hessus rotavirus-mediated perinatal hepatic inflammation. Sci Rep. 2020 Apr 28;10(1):7165 [PubMed: 32346042]

5. Imhof BA, Jemelin S, Ballet R, Vesin C, Schapira M, Karaca M, et al. CCN1/CYR61-mediated meticulous patrolling by Ly6Clow monocytes fuels vascular inflammation. Proc Natl Acad Sci. 2016 Aug 16;113(33):E4847–56. [PubMed: 27482114]

6. Guilliams M, Mildner A, Yona S. Developmental and Functional Heterogeneity of Monocytes. Immunity. 2018 16;49(4):595–613. [PubMed: 30332628]

7. Finsterbusch M, Hall P, Li A, Devi S, Westhorpe CLV, Kitching AR, et al. Patrolling monocytes promote intravascular neutrophil activation and glomerular injury in the acutely inflamed glomerulus. Proc Natl Acad Sci U S A. 2016 30;113(35):E5172–5181. [PubMed: 27528685]

8. Wang J-X, Bair AM, King SL, Shnayder R, Huang Y-F, Shieh C-C, et al. Ly6G ligation blocks recruitment of neutrophils via a β2-integrin–dependent mechanism. Blood. 2012 Aug 16;120(7):1489–98. [PubMed: 22661700]

9. Mack M, Cihak J, Simonis C, Luckow B, Proudfoot AE, Plachý J, et al. Expression and characterization of the chemokine receptors CCR2 and CCR5 in mice. J Immunol Baltim Md 1950. 2001 Apr 1;166(7):4097–704.

10. Cederblad L, Rosengren B, Ryberg E, Hermansson N-O. AZD8797 is an allosteric non-competitive modulator of the human CX3CR1 receptor. Biochem J. 2016 Mar 1;473(5):641–9. [PubMed: 26656484]

11. Ridderdstad Wollberg A, Ericsson-Dahlstrand A, Jurėus A, Ekerot P, Simon S, Nilsson M, et al. Pharmacological inhibition of the chemokine receptor CX3CR1 attenuates disease in a chronic-relapsing rat model for multiple sclerosis. Proc Natl Acad Sci U S A. 2014 Apr 8;111(14):5409–14. [PubMed: 24706865]

12. Karlström S, Nordvall G, Söhn D, Hettman A, Turek D, Åhlin K, et al. Substituted 7-Amino-5-thio-thiazolo[4,5-d] pyrimidines as Potent and Selective Antagonists of the Fractalkine Receptor (CX3CR1). J Med Chem. 2013 Apr 25;56(8):3177–90. [PubMed: 23516963]

13. Peiseler M, Kubes P. More friend than foe: the emerging role of neutrophils in tissue repair. J Clin Invest. 2019 Jul 1;129(7):2029–39. [PubMed: 30958798]

14. Frodermann V, Nahrendorf M. Neutrophil–macrophage cross-talk in acute myocardial infarction. Eur Heart J. 2017 Jan 14;38(3):198–200. [PubMed: 28158564]

15. Cossío I, Lucas D, Hidalgo A. Neutrophils as regulators of the hematopoietic niche. Blood. 2019 May 16;133(00320):2140–8. [PubMed: 30898859]
16. Horckmans M, Ring L, Duchene J, Santovito D, Schloss MJ, Drechsler M, et al. Neutrophils orchestrate post-myocardial infarction healing by polarizing macrophages towards a reparative phenotype. Eur Heart J. 2017 Jan 14;38(3):187–97. [PubMed: 28158426]

17. Zemans RL, Briones N, Campbell M, McClendon J, Young SK, Suzuki T, et al. Neutrophil transmigration triggers repair of the lung epithelium via beta-catenin signaling. Proc Natl Acad Sci U S A. 2011 Sep 20;108(38):15990–5. [PubMed: 21880956]

18. Wilgus TA, Roy S, McDaniel JC. Neutrophils and Wound Repair: Positive Actions and Negative Reactions. Adv Wound Care. 2013 Sep;2(7):379–88.

19. Kurimoto T, Yin Y, Haboub G, Gilbert H-Y, Li Y, Nakao S, et al. Neutrophils Express Oncomodulin and Promote Optic Nerve Regeneration. J Neurosci. 2013 Sep 11;33(37):14816–24. [PubMed: 24027282]

20. Wang J Neutrophils in tissue injury and repair. Cell Tissue Res. 2018 Mar;371(3):531–9. [PubMed: 29383445]

21. Paris AJ, Liu Y, Mei J, Dai N, Guo L, Spruce LA, et al. Neutrophils promote alveolar epithelial regeneration by enhancing type II pneumocyte proliferation in a model of acid-induced acute lung injury. Am J Physiol Lung Cell Mol Physiol. 2016 01;311(6):L1062–75. [PubMed: 27694472]

22. Theilgaard-Mönch K, Knudsen S, Follin S, Borregaard N. The Transcriptional Activation Program of Human Neutrophils in Skin Lesions Supports Their Important Role in Wound Healing. J Immunol. 2004 Jun 15;172(12):7684–93 [PubMed: 15187151]

23. Ashcroft GS, Lei K, Jin W, Longenecker G, Kulkarni AB, Greenwell-Wild T, et al. Secretary leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing. Nat Med. 2000 Oct;6(10):1147–53. [PubMed: 11017147]

24. Lörchner H, Pöling J, Gajawada P, Hou Y, Polyakova V, Kostin S, et al. Myocardial healing requires Reg3β-dependent accumulation of macrophages in the ischemic heart. Nat Med. 2015 Apr;21(4):353–62. [PubMed: 25751817]

25. Jaeschke H, Farhood A, Smith CW. Neutrophils contribute to ischemia/reperfusion injury in rat liver in vivo. FASEB J. 1990;4(15):3355–9. [PubMed: 2253850]

26. Xu R, Huang H, Zhang Z, Wang F-S. The role of neutrophils in the development of liver diseases. Cell Mol Immunol. 2014 May;11(3):224–31. [PubMed: 24633014]

27. Liu Z-X, Han D, Gunawan B, Kaplowitz N. Neutrophil depletion protects against murine acetaminophen hepatotoxicity. Hepatology. 2006;43(6):1220–30. [PubMed: 16729305]

28. Wang J, Hossain M, Thanabalasuriar A, Gunzer M, Meininger C, Kubes P. Visualizing the function and fate of neutrophils in sterile injury and repair. Science. 2017 Oct 6;358(6359):111–6. [PubMed: 28983053]

29. Alvarenga DM, Mattos MS, Lopes ME, Marchesi SC, Araújo AM, Nakagaki BN, et al. Paradoxical Role of Matrix Metalloproteinases in Liver Injury and Regeneration after Sterile Acute Hepatic Failure. Cells. 2018 Dec;7(12):247.

30. Sajjou E, Enomoto Y, Matsuda M, Kok CY-Y, Akira S, Tanaka M, et al. Neutrophils alleviate fibrosis in the CCl4-induced mouse chronic liver injury model. Hepatol Commun. 2018;2(6):703–17. [PubMed: 29881822]

31. Harty MW, Muratore CS, Papa EF, Gart MS, Ramm GA, Gregory SH, et al. Neutrophil Depletion Blocks Early Collagen Degradation in Repairing Cholestatic Rat Livers. Am J Pathol. 2010 Mar;176(3):1271–81. [PubMed: 20110408]

32. Gao S, Liu F. Fetal liver: an ideal niche for hematopoietic stem cell expansion. Sci China Life Sci. 2018 Aug 1;61(8):885–92. [PubMed: 29934917]

33. Shao L, Chang J, Feng W, Wang X, Williamson EA, Li Y, et al. The Wave2 scaffold Hem-1 is required for transition of fetal liver hematopoiesis to bone marrow. Nat Commun. 2018 Jun 18;9(1):2377. [PubMed: 29915352]

34. Yamamoto K, Miwa Y, Abe-Suzuki S, Abe S, Kirimura S, Onishi I, et al. Extramedullary hematopoiesis: Elucidating the function of the hematopoietic stem cell niche (Review). Mol Med Rep. 2016 Jan 1;13(1):587–91. [PubMed: 26648325]

35. Kim CH. Homeostatic and pathogenic extramedullary hematopoiesis. J Blood Med. 2010 Mar 23;1:13–9. [PubMed: 22282679]
36. Wolber FM, Leonard E, Michael S, Orschell-Traycoff CM, Yoder MC, Srour EF. Roles of spleen and liver in development of the murine hematopoietic system. Exp Hematol. 2002 Sep 1;30(9):1010–9. [PubMed: 12225792]

37. Mumau MD, Vanderbeck AN, Lynch ED, Golec SB, Emerson SG, Punt JA. Identification of a Multipotent Progenitor Population in the Spleen That Is Regulated by NR4A1. J Immunol Baltim Md 1950.2018 01;200(3):1078–87.

38. Ryu SH, Na HY, Sohn M, Choi W, In H, Shin H Soo, et al. Identification of splenic progenitors for CD11b high myeloid cells in the long-term culture with GM-CSF. J Immunol. 2019 May 1;202(1 Supplement):118.4–118.4.