

## **The inflammasome: more than a protective innate immune mechanism**

Bilyana Stoilova<sup>1</sup> and Paresh Vyas<sup>1,2</sup>

<sup>1</sup>MRC MHU, BRC Hematology Theme, Oxford Biomedical Research Centre, Oxford Centre for Haematology, WIMM, Radcliffe Department of Medicine, University of Oxford, UK OX3 9DU. <sup>2</sup>Department of Haematology, Oxford University Hospitals NHS Trust OX3 7LE.

Word count excluding references: 944 words.

**Little is known about the inflammasome beyond its function in innate immune response. In this issue of Immunity, Tyrkalska et al. report that the inflammasome regulates the balance between erythroid and myeloid differentiation in model systems, which could provide new insights into hematopoietic lineage bias associated with inflammatory conditions.**

The inflammasome coordinates cellular immune homeostasis, cell metabolism, proliferation, transcriptional response to a broad range of microbial motifs, endogenous danger signals and environmental irritants and its therapeutic targeting might have important clinical implications (Van Opdenbosch and Lamkanfi, 2019). The inflammasome consists of sensors (nucleotide binding domain and leucine rich repeat gene family proteins, NLRs), adaptor proteins (for example, ASC, apoptosis-associated speck-like proteins containing caspase recruitment domains or CARDs,) and effector proteins (for example, caspase 1), which regulate the self-cleavage and conversion of inactive zymogen form of effector proteins into their active forms. Activation of caspase-1 results in proteolytic cleavage of the inactive forms of pro-inflammatory cytokines interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-18, into their active forms and the induction of a lytic, inflammatory form of programmed cell death called pyroptosis, which augments inflammatory responses. In humans a range of monogenic autoimmune and inflammatory diseases that can also be cancer-predisposing, have been causally linked to germline variants in genes encoding components of the inflammasome (reviewed in (Van Opdenbosch and Lamkanfi, 2019).

Chronic inflammatory diseases, aging and some clonal human preleukemic conditions like myelodysplasia (MDS) are associated with a bias toward the myeloid hemopoietic lineage (usually the granulocyte-macrophage or GM lineage) and anemia. The mechanisms for these changes are likely to be multiple and complex. For example, hemopoietic cell autonomous mechanisms likely operate as acquired mutations in genes regulating hemopoiesis and lineage bias are seen in clonal hemopoiesis (Genovese et al., 2014; Jaiswal et al., 2014; Xie et al., 2014) and MDS (Papaemmanuil et al., 2013). However, some of these mutations also alter mature monocyte function causing an inflammatory milieu (Fuster et al., 2017) suggesting an interplay of cell-autonomous and non-cell autonomous mechanisms controlling lineage bias and inflammation during aging. Finally, overexpression of inflammasome proteins has been documented in MDS and been associated with pyroptosis (inflammatory cell death). Knockdown or pharmacological inhibition of the inflammasome in MDS models and cells restored more effective hematopoiesis, suggesting that the inflammasome could be a therapeutic target in MDS (Basiorka et al., 2016).

In this issue of *Immunity*, Tyrkalska et al. (2019) report a previously unknown function of the inflammasome to regulate the balance between erythroid and myeloid differentiation in hemopoiesis. Using zebrafish larvae as a model, the authors showed that genetic inhibition of two inflammasome components (Gbp4 and Asc) or pharmacological inhibition of caspase-1 significantly decreases neutrophil and macrophage myeloid cells. Conversely, simultaneous expression of Asc and the functional homolog of mammalian caspase-1 Caspa, significantly increased the number of myeloid cells. Concurrently, genetic and pharmacological inflammasome inhibition increased erythrocyte abundance. The authors further demonstrate the inflammasome's role not only in steady-state but during emergency infection-driven myelopoiesis. Taken together, the inflammasome regulates the balance between myeloid and erythroid differentiation. No effect was seen on emergence of definitive hematopoietic stem and progenitor cells.

To shed light on molecular mechanisms the authors studied expression of two master hemopoietic lineage regulators – the erythroid transcription factor GATA1 and the granulocyte-monocyte myeloid transcription factor PU.1 (SPI1) that each promote the differentiation of the two lineages they are expressed in. Human caspase-1, CASP1, was able to directly cleave human GATA1 *in vitro*, suggesting that the inflammasome may directly suppress erythropoiesis by GATA1 cleavage and inactivation. Inhibition of Casp1 in mouse haematopoietic stem cells (HSCs) upregulated Gata1 protein and increased megakaryocyte-erythrocyte colony output and concordantly decreased granulocyte-monocyte colony formation, suggestive of an evolutionarily conserved mechanism regulating granulocyte-monocyte and erythroid lineages by the inflammasome. Similar GATA1 cleavage mechanism by caspases downstream of death receptor signaling has been previously shown to affect erythroid differentiation in human erythroid progenitors (De Maria et al., *Nature*, 1999). Interestingly, in differentiating mouse HSCs inflammasome inhibition affected only GATA1 levels but not PU.1 levels, suggesting any effect on PU.1 may be indirect.

In order to show the relevance of their finding to disease contexts the authors studied two zebrafish models; one of the chronic neutrophilic inflammatory dermatosis and of Diamond-Blackfan anemia. In neutrophilic dermatosis, a disease characterized by the large infiltration of neutrophils in the skin in the absence of infection, pharmacological inhibition or genetic inactivation of caspase-1 reduced neutrophilia but without abrogating neutrophil skin infiltration. Similarly, in a zebrafish larvae model of Diamond-Blackfan anemia, a rare ribosomopathy resulting in reduction in GATA1 translation and reduced red blood cell production, the reversible pharmacological inhibition of caspase-1 partially rescued hemoglobin defects and decreased Spi1/Gata1 protein ratio. Taken together, the pharmacological inhibition of caspase-1 and manipulation of the inflammasome might be an attractive new target in human chronic inflammatory diseases and anemia. Importantly, a number of inflammasome inhibitors are in clinical practice or are in development (Mangan et al., 2018).

Like all good studies, though these observations are encouraging, they raise questions. The precise molecular mechanisms controlling hemopoietic lineage bias still remain to be determined. Is the major impact of the inflammasome on GATA1 and PU.1 proteins levels or are other regulators, such as cytokines or signalling proteins, direct or indirect targets of the inflammasome. From a cellular perspective, it will be important to understand which hemopoietic progenitor and precursor cells are affected by these molecular mechanisms. For example, there are GATA1-expressing myeloid cells that contribute to specific inflammatory processes like mast cells and eosinophils (Drissen et al., 2019). Would targeting the inflammasome affect levels and function of these cell types? Lastly, the role of a complicated multi-layered processes generating and sustaining the inflammasome is likely to vary from specific disease to disease context. The work from Tyrkalska et al. (2019) is likely stimulate more others to tackle these, and other questions, on the intersection of the inflammasome and hemopoietic cell output.

## References

- Basiorka, A.A., McGraw, K.L., Eksioglu, E.A., Chen, X., Johnson, J., Zhang, L., Zhang, Q., Irvine, B.A., Cluzeau, T., Sallman, D.A., *et al.* (2016). The NLRP3 inflammasome functions as a driver of the myelodysplastic syndrome phenotype. *Blood* 128, 2960-2975.
- Drissen, R., Thongjuea, S., Theilgaard-Monch, K., and Nerlov, C. (2019). Identification of two distinct pathways of human myelopoiesis. *Sci Immunol* 4.
- Fuster, J.J., MacLauchlan, S., Zuriaga, M.A., Polackal, M.N., Ostriker, A.C., Chakraborty, R., Wu, C.L., Sano, S., Muralidharan, S., Rius, C., *et al.* (2017). Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science* 355, 842-847.
- Genovese, G., Kahler, A.K., Handsaker, R.E., Lindberg, J., Rose, S.A., Bakhoum, S.F., Chambert, K., Mick, E., Neale, B.M., Fromer, M., *et al.* (2014). Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* 371, 2477-2487.
- Jaiswal, S., Fontanillas, P., Flannick, J., Manning, A., Grauman, P.V., Mar, B.G., Lindsley, R.C., Mermel, C.H., Burt, N., Chavez, A., *et al.* (2014). Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 371, 2488-2498.
- Mangan, M.S.J., Olhava, E.J., Roush, W.R., Seidel, H.M., Glick, G.D., and Latz, E. (2018). Targeting the NLRP3 inflammasome in inflammatory diseases. *Nat Rev Drug Discov* 17, 588-606.

Papaemmanuil, E., Gerstung, M., Malcovati, L., Tauro, S., Gundem, G., Van Loo, P., Yoon, C.J., Ellis, P., Wedge, D.C., Pellagatti, A., *et al.* (2013). Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood* 122, 3616-3627; quiz 3699.

Tyrkalska, S., Pérez-Oliva, AB., Rodríguez-Ruiz, L., Martínez-Morcillo, F., Alcaraz-Pérez, F., Martínez-Navarro, F., Lachaud, C., Ahmed N., Schroeder, T., Pardo-Sánchez, I., Candel, S., López-Muñoz, A., Choudhuri, A., Rossmann, M., Zon, L., Cayuela, M., García-Moreno, D., Mulero, V. Inflammasome regulates hematopoiesis through cleavage of the master erythroid transcription factor GATA1 (this issue).

Van Opdenbosch, N., and Lamkanfi, M. (2019). Caspases in Cell Death, Inflammation, and Disease. *Immunity* 50, 1352-1364.

Xie, M., Lu, C., Wang, J., McLellan, M.D., Johnson, K.J., Wendl, M.C., McMichael, J.F., Schmidt, H.K., Yellapantula, V., Miller, C.A., *et al.* (2014). Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med* 20, 1472-1478.