RESEARCH HIGHLIGHT

NO-immune privilege for hematopoietic stem cells

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Hematopoietic stem cells (HSCs) are localized in anatomically and functionally distinctive bone marrow (BM) niches that regulate their regenerative functions in hematopoiesis and immunity; yet the cellular and molecular underpinnings of these niches remain incompletely defined. A recent study in *Nature* highlighted nitric oxide as a regulator of dormant, self-renewing HSCs that reside in immune-privileged vascular niches near the bone surface (endosteal) in the metaphyseal BM.

All hematological and immune cells in our body can be derived from a common multipotent ancestor — the hematopoietic stem cell (HSC). A paradigmatic demonstration of this potential is HSC transplantation (HSCT), which regenerates the hematopoietic and immune systems of the recipient from donor-derived HSCs. Allogeneic HSCT (allo-HSCT) is used in the clinic to treat hematological malignancies and inherited immune, metabolic or bone marrow (BM) failure syndromes. However, healthy allogeneic HSCs must remain protected from immune attack to sustain long-term hematopoiesis.

HSCs reside in specialized BM microenvironments called "niches", which regulate their self-renewal, commitment, differentiation and migration. Cumulative research over the last decades suggests a high degree of specialization in the HSC niches to fulfill the requirements of homeostatic and regenerative hematopoiesis. The specialization is supported by anatomical, cellular, molecular and physiological evidence. A smaller endosteal/paratrabecular niche comprising capillaries, arterioles and associated mesenchymal stromal cells (MSCs) promotes HSC quiescence and lymphopoiesis, and has been postulated as essential for regenerative (not homeostatic) hematopoiesis. By contrast, a much larger non-endosteal/non-paratrabecular/central BM comprising sinusoids permissive for hematopoietic cell transmigration harbors the majority of active HSCs and facilitates myelopoiesis.[1]

Different cellular components of these niches are involved in HSC regulation. Among them, regulatory T (T_{reg}) cells were previously shown to protect HSCs from immune attack and promote their persistence after allo-HSCT.[2] Since long-term HSC tolerance is key to a satisfactory outcome of allo-HSCT, characterizing this immune-privileged HSC niche has a significant scientific and clinical interest.

In their recent publication, Furuhashi et al.[3] describe endosteal capillaries displaying primary ciliated endothelium and high expression of the immune checkpoint molecule CD200. The ciliary protein IFT20 was found necessary to induce CD200, which in turn increases production of nitric oxide (NO) in HSCs through CD200 receptor-dependent activation of endothelial NO synthase (eNOS). In HSCs, eNOS-derived NO modulates autophagy and supports HSC dormancy, self-renewal and immune protection. Overall, these novel findings confirm the important role of NO in HSC maintenance as previously described[4] and expand it to immune protection. They also expand the regulatory role of primary cilia from embryonic HSCs in zebrafish[5] to adult mammalian HSCs, and add further evidence for mechano-regulation of hematopoiesis.[6]

First, Furuhashi et al. noted that allogeneic HSCs from B6 donor mice persisting in the BM of non-conditioned immunocompetent BALB/c mice were associated with vessels displaying a distinct sharp hairpin curve-like architecture. They hypothesized that high sheer stress in these vessels might locally raise the level of NO, which had been previously demonstrated to be an important regulator of HSC engraftment, maintenance and self-renewal.[4] Indeed, NO^{hi} HSCs were found to be enriched near these curved endosteal vessels. Further characterization of NO^{hi} HSCs revealed their quiescence and high expression of eNOS and immunomodulatory molecules, such as CD200 receptor. Competitive serial transplantation in mice enabled the authors to reveal the existence of a late-rising pattern compatible with highly dormant HSCs, and an unbiased hematopoietic lineage reconstitution from NO^{hi} HSCs, suggesting that high NO levels mark dormant HSCs with a high self-renewal potential. A previous study highlighted the importance of balanced NO regulation for HSC maintenance, but concluded instead that BM self-renewing HSCs exhibit lower NO levels compared with circulating HSCs with reduced self-renewal.[4] Since migratory HSCs are enriched near BM sinusoids (which are permeable for transmigration), whereas Furuhashi et al. found NO^{hi} HSCs near endosteal capillaries, it is possible that NO-dependent HSC regulation differs in these distinct vascular niches.

Some studies have suggested that primary cilia regulate HSC emergence during development in zebrafish[5] and might be involved in the mechano-regulation of hematopoiesis.[6] However, the role of primary cilia in adult hematopoiesis is largely unexplored. Furuhashi et al. used cilia reporter mice to describe the presence of ciliated structures that were virtually restricted to $CD200^{hi}$ capillaries; this was further confirmed through electron microscopy, highlighting a previously unrecognized feature of endosteal capillaries.

To interrogate their function, the authors carried out a series of elegant functional studies. These included conditional deletions of the ciliary protein IFT20 or the immunoregulatory protein CD200 in endothelial cells, or selective elimination of eNOS in HSCs. These

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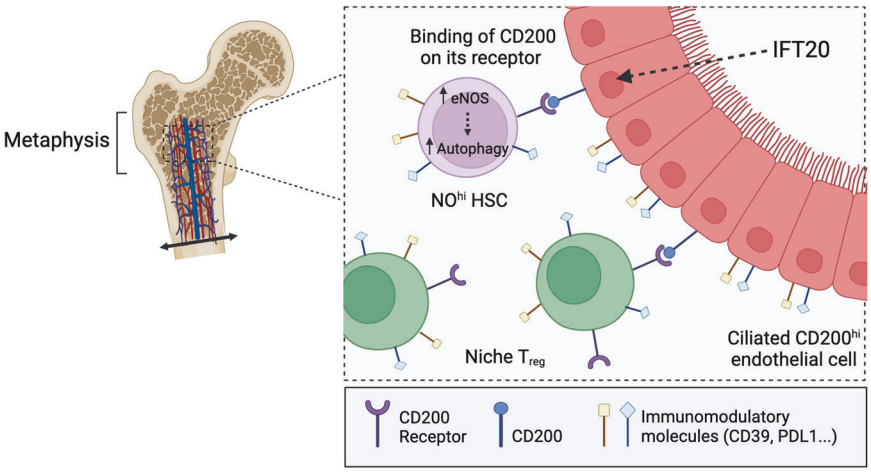

Fig. 1 Model of NO-mediated immuno-protection of HSCs. High expression of NO defines highly primitive and immune-protected HSCs located near endosteal ciliated CD200^{hi} vessels, which are abundant in the metaphyseal BM. IFT20-CD200-eNOS-autophagy axis has been identified as the regulator of NO^{hi} HSC quiescence and late-rising potential. High levels of immunomodulatory molecules produced by CD200^{hi} endothelial cells as well as their recruited T_{reg} cells protect NO^{hi} HSCs against immune attack, promoting their persistence after allo-HSCT. The figure is created with BioRender.

loss-of-function experiments caused similar reductions of BM cellularity, HSCs (including NO^{hi} HSCs), eNOS expression and autophagy regulators, leading to impaired long-term hematopoietic reconstitution. The authors concluded that the ciliary protein IFT20 is required to induce CD200, which in turn increases NO production in HSCs via CD200 receptor-dependent activation of eNOS. Altogether, the IFT20-CD200-eNOS-autophagy axis emerges as a novel regulator of NO^{hi} HSCs in the endosteal niche (Fig. 1).

Finally, the authors investigated the possible immunoregulatory role of metaphyseal CD200^{hi} capillaries for NO^{hi} HSCs. They suggest that CD200^{hi} endosteal capillaries might provide immune protection to allogeneic NO^{hi} HSCs by expressing high levels of immunomodulatory molecules, such as CD39 and PD-L1, and by recruiting T_{reg} cells.

The authors developed a high-resolution three-dimensional two-photon confocal microscopy of long bones to visualize NO^{hi} HSCs within their native BM microenvironment. They identified a thin capillary network (< 3–4 μm in diameter) with high expression of CD200 particularly enriched in the metaphyseal BM. Intriguingly, these capillaries appeared to be different from other small-caliber vessels found in the same region, such as transition zone vessels containing endomucin-high endothelium,[7] which was found instead in closer proximity to NO^{low} HSCs. This finding suggests that NO levels might allow a higher degree of discrimination of BM microdomains supporting primitive HSCs, but also raises interesting questions for future research: 1) Some CD200^{hi} cells appear to surround transition zone vessels; what is the relationship between CD200^{hi} capillaries and transition zone vessels? 2) The latter vessels are reduced during aging,[8–10] which is associated with decreased lymphopoiesis. Is there a parallel alteration of CD200^{hi} capillaries and does this influence HSC immune protection with age? 3) Furuhashi et al. found neuronal NOS (nNOS) expression in HSCs, and nNOS in the niche (MSCs) has been involved in reduced lymphopoiesis during aging.[10] Is there a role for nNOS in HSCs or their niche in regulating hematopoiesis? 4) More broadly, NO can be exchanged between HSCs and their niches, adding another later complexity in NO-dependent hematopoietic regulation.

Overall, this thought-provoking article has implications for the mechanobiology of hematopoiesis and suggests new potential therapeutic targets to improve reconstitution and immune tolerance after allo-HSCT.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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