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SPOTLIGHT

The staying power of hematopoietic stem cells

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Hematopoietic stem and progenitor cells (HSPCs) use specialized adhesive structures referred to as magnupodium to stay in hematopoietic niches. Bessey et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202005085) define new characteristics of the magnupodium, including centriole polarization and the necessary and sufficient role of CXCR4 signaling.

Hematopoietic stem and progenitor cells (HSPCs) need to stay in osteal and vascular niches in the bone marrow in the face of a massive flux of differentiating and maturing cells that might compete with or displace HSPCs. There have been only glimpses of the nature of the adhesive connection between HSPCs and the key niche cells. Bessy et al. use in vitro coculture systems with HSPCs and osteoblasts and/or endothelial cells, in conjunction with 3D immunofluorescence imaging, to define the nature of this critical cell–cell interface (1). In a microfluidic platform that provides HSPCs with a choice between osteoblasts and endothelial cells to establish a niche, Bessy et al. found that that HSPCs migrate into the synthetic osteal and vascular compartments and establish polarized interactions based on positioning of the centrioles toward the niche cell in the majority of observations. Bessy et al. propose that HSPCs form a specialized junction with niche cells characterized by polarity, adhesion, and stability, hallmarks of established synapses (2).

Previous reports have focused on the role of a variety of cell projections in HSPC interactions with niche cells, including nanotubes many cell diameters in length and more substantial protrusions containing actinomyosin contractile systems that doubled the cell length. The latter appeared similar to the uropod of a migrating cell, except that it was attached at one end to the niche cell. Both types of structures were referred to as magnupodium due to their great relative dimensions. In microwell-based 1:1 coculture of HSPCs and osteoblasts, Bessy et al. clearly observe substantial protrusion emerging from HSPCs that are stably anchored to the osteoblast surface (1), which they refer to as magnupodium to follow the earlier precedents. The magnupodium allows the HSPC cell body to actively pivot about the stable attachment site, extensively surveying the 3D niche adjacent to the osteoblast while remaining firmly attached (1). While nanotubes and uropods are typically thought of as trailing structures in relation to cell migration (3), Bessy et al. conceptualize the magnupodium as the forward-facing synaptic structure of the niche bound HSPC; the dynamic cell body is viewed as the "tail" in this setting. This is an exciting inversion of the typical conventions and creates a framework for asking new questions and comparison to other synaptic systems.

The HSPC niche requires the chemokine receptor CXCR4 on the HSPC and the chemokine SDF-1 on the stromal cell to establish a functional niche (4). Magnupodium formation with centriolar polarization is completely dependent upon CXCR4 function (Fig. 1; 1). They further demonstrate that polarized magnupodium formation by HSPCs is highly specific to bone marrow stromal cells, as HSPCs did not form a magnupodium with fibroblasts, and mature hematopoietic cells don't form polarized magnupodium on osteoblasts. Importantly, the centrioles are not only oriented toward the magnupodium but are fully dissociated from the nucleus and positioned near to the tip of the magnupodium in contact with the niche cell. This is a critical point as docking of the centrioles directly at the synaptic cleft is a hallmark of immunological synapses (5, 6). The proteins enriched in the HSPC magnupodium include typical uropod proteins like ezrin and phosphorylated myosin II light chain. In contrast, CD44, which is often found in the uropod of migrating cells, is localized to the HSPC tail. CD133, lymphocyte function associated-1 (LFA-1), very late activation-4, the Arp2/3 complex, and the Golgi apparatus are found near the tip of magnupodium, consistent with synaptic adhesion and directed secretion. Thus, Bessy et al. strengthen the case that the magnupodium is a new type of communication structure that defines the HSPC niche. It will be important to directly test the potential for polarized secretion through the magnupodium to further investigate the synapse analogy.

The magnupodium has implications for work on immune cell communication beyond HSPCs. Bessy et al. show that T cells and monocytes will not form a magnupodium-like structure with osteoblasts, which makes sense to avoid competition with HSPCs in the bone marrow, but this is not to say that a mature lymphocyte could never form a magnupodium in a relevant biological context. Early work with purified adhesion molecules revealed magnupodium-like structures

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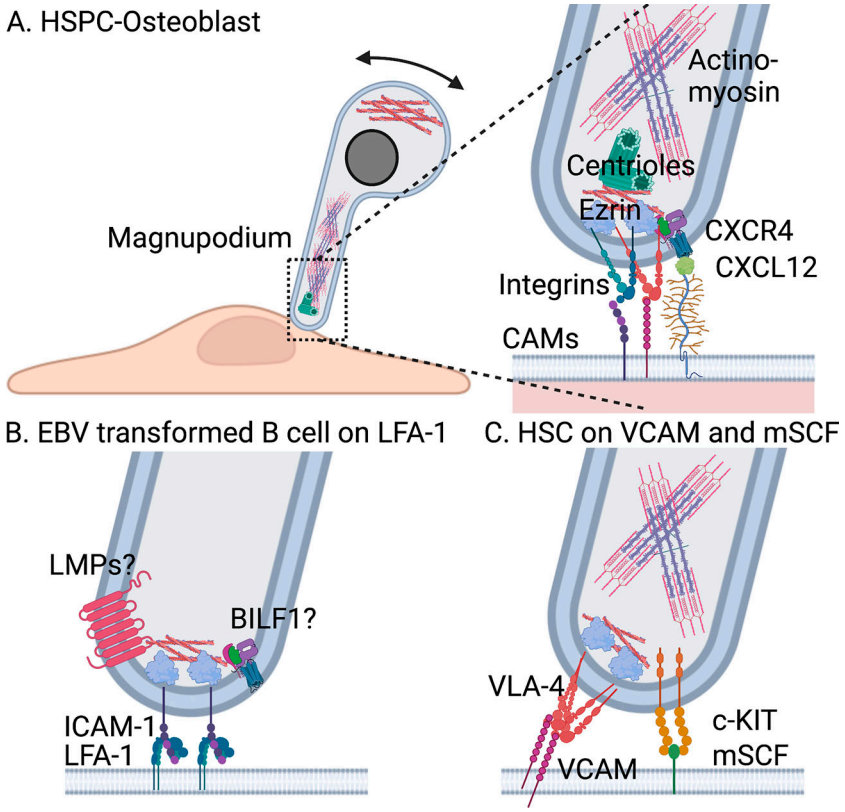

Figure 1. **Magnupodium formation between HSPCs and osteoblasts and reductionist models. (A)** Schematic of HSPC formation of a magnupodium with an osteoblast and potential receptor ligand interactions and cytoskeletal machinery. **(B)** Magnupodium-like structure formed by EBV transformed B cell on purified LFA-1. LMP, latent membrane protein (7). **(C)** Magnupodium formation in response to laterally mobile VCAM and mSCF (10).

formed by Epstein-Barr virus (EBV) transformed B lymphocytes when attached to supported lipid bilayers (SLBs) presenting purified LFA-1 (Fig. 1 B; 7). Time-lapse imaging revealed that the lymphoblasts were anchored in place for up to 16 h, the longest observation period (7). This is more durable than an antigen dependent immunological synapse (8). In light of results from Bessy et al., it's surprising that an activated B cell line could form such a complex structure in response to engaging a single adhesion molecule. However, EBV encodes constitutively active surface receptors that mimic antigen receptor tyrosine kinase cascades, TNF receptor associated factor activation of NF-κB, and chemokine signaling (9). More recently, Hao et al. used SLBs containing laterally mobile vascular cell adhesion molecule (VCAM) and membrane anchored stem cell factor (mSCF) to trigger formation of a magnupodium-like structure by HSPCs (Fig. 1 C; 10). Hao et al. presented SDF1 in a laterally mobile form on an SLB and found that this presentation is not sufficient for magnupodium-like structure formation, whereas Bessy et al. presented SDF1 anchored to a solid surface and found that it is sufficient for magnupodium formation. This suggests that that the way in which SDF1 is presented on the osteoblast or endothelial cell, which could include the number of molecules per unit area or the lateral mobility, may be important for magnupodium formation. c-KIT signaling, triggered by mSCF, is similar in some respects to antigen receptor signaling in B cells. Neither of these studies investigated whether the centrioles are positioned near the protein-presenting SLB, which is critical to establishing if these structures are magnupodia according to the criteria proposed by Bessy et al. These SLB-based model systems should be helpful to further dissect signals sufficient for magnupodium formation.

There could also be at least one physiological role for the magnupodium in B cell. Early in T cell help for antibody production by B cells, the antigen specific T and B cells meet at the boundary between the T cell zone and B cell follicle in secondary lymphoid tissues. The B cell and T cell do an intimate dance in which the B cell leads (11). The B cell migrates at ∼5 µm/min while dragging the T cell through the immunological synapse. In light of Bessy et al., it would be important to ask if the B cell side of the B–T immunological synapse is a magnupodium, creating a two-way communication interface in which both cells are polarized toward each other, even as one migrates in response to chemokine gradients. The criteria established by Bessy et al. could now be applied to the B–T synapse established in an appropriately engineered tissue-like environment. Depending upon the outcome of such experiments, this may suggest a potential for multitasking between motility directed by the leading lamellipodium and a synaptic interface through a magnupodium formed by the same cell.

This concept of multitasking may also apply to HSPCs. HSPCs need to monitor many inputs to self-renew, differentiate, or even mobilize during inflammation. In polarized T cells, the free lamellipodium is much more sensitive than the uropod to antigen presenting cells (12). The magnupodium provides a solid connection between cells across which to exchange information over long periods, but the active waving by the HSPC tail in the 3D niche may provide opportunities to sense additional contact-dependent and/or soluble signals. In this regard it may be relevant that CD44 is part of this tail complex of the HSPC. CD44 is a critical sensor and regulator of inflammation (13), and it's possible that it can better monitor the status of the niche from the tail than from the magnupodium, which is likely more focused on the state of the niche cell and providing the HSPC with its power to stay in the niche.

In summary, Bessy et al. provide the field with a new perspective on the HSPC niche and help to explain the remarkable staying power and flexibility of HSPCs.

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References

1. Bessy, T., et al. 2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202005085
2. Dustin, M.L., and D.R. Colman. 2002. *Science.* https://doi.org/10.1126/science.1076386
3. Önfelt, B., and D.M. Davis. 2004. *Biochem. Soc. Trans.* https://doi.org/10.1042/BST0320676
4. Nie, Y., et al. 2008. *J. Exp. Med.* https://doi.org/10.1084/jem.20072513
5. Cassioli, C., et al. 2021. *J. Cell Sci.* https://doi.org/10.1242/jcs.258462
6. Douanne, T., and G.M. Griffiths. 2021. *Curr. Opin. Cell Biol.* https://doi.org/10.1016/j.ceb.2021.02.008
7. Dustin, M.L., et al. 1992. *J. Immunol.* 148:2654–2663.
8. Mayya, V., et al. 2018. *Cell Rep.* https://doi.org/10.1016/j.celrep.2017.12.052
9. Tsutsumi, N., et al. 2021. *Immunity.* https://doi.org/10.1016/j.immuni.2021.06.001
10. Hao, J., et al. 2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202010118
11. Allen, C.D., et al. 2007. *Science.* https://doi.org/10.1126/science.1136736
12. Negulescu, P.A., et al. 1996. *Immunity.* https://doi.org/10.1016/S1074-7613(00)80409-4
13. Teder, P., et al. 2002. *Science.* https://doi.org/10.1126/science.1069659