Comment on Mayack and Wagers, page 519

Osteoblasts: yes, they can

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In this issue of Blood, Mayak and Wagers report on a new method to isolate putative osteoblastic cells that can regulate hematopoietic stem cell proliferation and function.

The cellular and extracellular matrix components that support and regulate hematopoietic stem cells (HSCs) form niches that localize near bone-lining osteoblasts (OBs)1,2 and blood vessels3,4 in the bone marrow (BM). However, the contribution of specific stromal-cell types to HSC maintenance remains unresolved because they have not yet been prospectively isolated and grown ex vivo.

Mayak and Wagers describe the purification by fluorescence-activated cell sorting of putative osteoblastic cells based on osteopontin (OPN) expression and the absence of hematopoietic markers CD45 and Ter119. Because OPN, a secreted and cell-surface molecule expressed in OBs, regulates HSC proliferation,5 Mayak and Wagers hypothesized that OPN+ cells represent the osteoblastic component of the niche.

In situations in which OBs are increased, the number of HSCs is concomitantly increased,1,2 suggesting that the number of available niches limits the size of the HSC pool in the BM. Mayak and Wagers reasoned that the reverse would also be true: in conditions where HSC numbers are increased, the number of niches may also increase.

Treatment with cyclophosphamide (Cy) and granulocyte colony-stimulating factor (G-CSF) induces HSC proliferation and subsequent mobilization into peripheral blood. The authors show that OPN+ cells proliferate in response to daily Cy+ G-CSF (Cy/G) injections; OPN+ numbers peaked at day 2 of Cy/G treatment, returning back to homeostasis at day 4. It is notable that OB proliferation was not seen when mice were mobilized with G-CSF alone, an observation consistent with OB suppression and down-regulation of the transcription factor Runx2.6 Given that G-CSF alone is a powerful mobilizer, this would argue that OB proliferation is a separate event that may not necessarily lead to actual mobilization of HSCs.

If OPN+ cells were bona fide niche components, they would be able to nurture and support HSCs. Hence, Mayak and Wagers purified OPN+ cells from untreated mice (UNT cells) or from 2 days of Cy/G treatment (D2 cells) and cocultured them for 12 hours with lineage-negative cells (a population enriched in HSCs). Analysis of c-kit-Thyl.1+Lineage-Scal+ cells (KTLS, a population highly enriched in HSCs revealed that UNT OPN+ cells limited KTLS expansion, but D2 cells greatly increased KTLS proliferation when compared with culture of Lineage- cells alone or with UNT cells. When the authors determined HSC activity in BM transplantation assays, they found more engraftment and reconstitution of UNT cells compared with cells incubated without OBs, indicating that UNT cells reduce HSC proliferation while simultaneously maintaining HSC function. In contrast, D2 cells greatly increased engraftment potential. Thus Cy/G treatment induces OPN+ cell proliferation and releases their inhibition on HSC growth while simultaneously maintaining their “stemness.”

Mayak and Wagers also show that ATM, a DNA repair molecule, is required for the expansion of OPN cells during Cy/G mobilization, and that Amph+/- D2 cells do not enhance HSC engraftment after coculture. As Cy is a DNA-damaging agent, and G-CSF alone does not induce OPN+ cell proliferation, these results suggest that the OB acts as a sensor of DNA damage, expanding the number of HSC niches and triggering HSC proliferation.

The main contribution of this work is the isolation and culture of putative osteoblastic cells that can regulate HSC function ex vivo. Although the coculture of OBs with HSCs in the present study is too short to determine whether they can expand HSCs, this method should permit further dissection of the mechanisms used by OBs to regulate HSC function. The ability to expand HSCs while maintaining their repopulating potential remains the holy grail of transplantation biology, and a first step for multiple therapeutic applications.

A greater understanding of the niche is probably the best approach toward this goal.

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REFERENCES

Comment on Wimmer-Kleikamp et al, page 721

PTPases: “Eph”ective arbitators of attraction

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In this issue of Blood, Wimmer-Kleikamp and colleagues report that high levels of cellular PTPases hold activities of EphA3 kinase in check and support stable cell-cell adhesion, rather than default repulsion.
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