

INSIGHTS

PreB cells are moving on

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In this issue of *JEM*, Fistonich et al. (<https://doi.org/10.1084/jem.20180778>) address how the bone marrow microenvironment supports diverse lineages through multiple developmental stages. Differential motility between pro- and preB cells results in differential IL-7 exposure, and, intriguingly, stromal cells respond to abnormal B cells by reducing *Il7*.

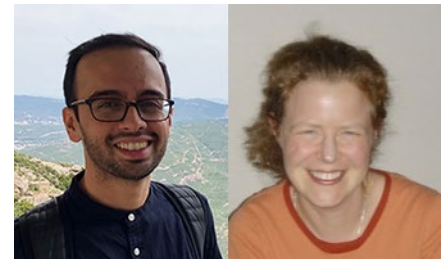
The bone marrow sustains hematopoietic stem cells and supports the development of several lineages of immune cells. How each of the diverse progenitor populations in the bone marrow obtains the unique combination of factors required to survive and differentiate remains a question of great interest (Hoggatt et al., 2016; Crane et al., 2017; Wei and Frenette, 2018). Here, Fistonich et al. show that while pro- and preB cells reside in similar areas of the bone marrow microenvironment, pro- and preB cells have substantially different motility. Fistonich et al. (2018) link differences in motility to differences in exposure to the cytokine IL-7. A defining aspect of hematopoiesis is that the developing cells are mobile, and these observations underscore the importance of considering the dynamics of cell-cell interactions in defining a hematopoietic cell's "niche." Intriguingly, while assessing the localization of preB cells that had sustained DNA damage while rearranging the B cell receptor (BCR), Fistonich et al. (2018) observed that stromal cells respond to B cells with unrepaired double-stranded DNA breaks by reducing *Il7* expression. In many cases, cancerous cells destroy the bone marrow niche for normal hematopoietic cells, and these findings will inspire closer investigation of how the niche may "fight back."

The story began with the puzzle that while both proB cells and preB cells depend on IL-7 signaling for survival and proliferation, in preB cells, IL-7 signaling also inhibits rearrangement of the Ig light chain (Clark et al., 2014). This suggested that as B cells transition to preB cells, they must somehow reduce their IL-7 exposure. An obvious hypothesis was that the two cell types reside in different parts of the bone marrow.

Fistonich et al. (2018) assessed the location of pro- and preB cells relative to a population of CXCL12^{hi}IL-7^{hi} cells, which they had previously characterized as a key component of the niche for hematopoietic stem cells and common lymphoid progenitors, and which express both a critical retention signal (CXCL12, a chemokine that binds the receptor CXCR4) and survival factor (IL-7) for developing B cells (Cordeiro Gomes et al., 2016). In fixed sections, the positioning of pro- and preB cells relative to CXCL12^{hi}IL-7^{hi} cells was similar, with the vast majority in contact with CXCL12^{hi}IL-7^{hi} stroma.

Using intravital two-photon microscopy, Fistonich et al. (2018) found that fixed sections concealed a striking difference between proB and preB cells. While proB cells engaged in relatively stable interactions with stromal cells, preB cells moved more rapidly through the bone marrow microenvironment. The faster movement of preB compared with proB cells correlated with a combination of increased CXCR4-mediated chemotaxis and, more striking, decreased $\alpha 4 \beta 1$ integrin-mediated adhesion. These results are nicely in line with findings that developing B cells transition from an adherent stage, in which they attach strongly to the OP9 stromal cell line in culture and are highly proliferative, to a nonadherent stage, in which genes enabling light chain recombination are expressed. The transcription factor Ikaros plays a key role in this transition by regulating genes involved in adhesion and motility (Joshi et al., 2014; Schwickert et al., 2014).

Fistonich et al. (2018) next asked what regulates the difference in motility between pro- and preB cells, and whether different migratory patterns result in different cyto-



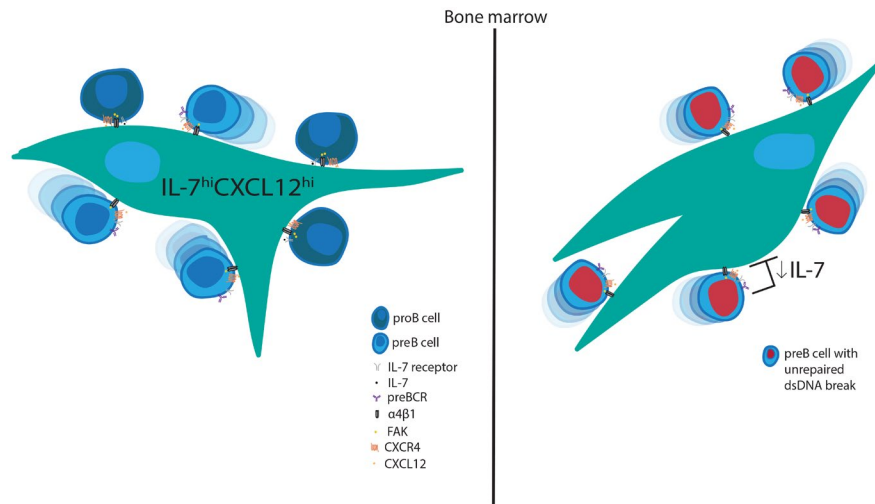
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kine exposure. They developed an elegant model to explain the switch. A positive feedback loop enforces proB cell interactions with IL-7^{hi}CXCL12^{hi} cells. IL-7 signaling in proB cells increases the activity of CXCR4 and $\alpha 4 \beta 1$ integrin; the resulting strengthened adhesion increases IL-7 exposure. PreBCR signaling breaks the cycle by simultaneously further increasing CXCR4 activity and decreasing integrin-mediated adhesion, which releases preB cells to move more quickly through the marrow and limits their IL-7 exposure. This model is challenging to test, because ideally it would require fine manipulation of cell adhesion at precise stages of B cell development. Nonetheless, the phenotypes of B cells upon IL-7 receptor blockade or treatment with BCR agonists were consistent with the model. IL-7 receptor signaling and preBCR signaling had particularly strong and opposing effects on focal adhesion kinase (FAK), a protein that is activated downstream of integrin ligation, promotes proB cell retention in the bone marrow, and is down-regulated in preB cells compared with proB cells (Park et al., 2013; Joshi et al., 2014; Schwickert et al., 2014). Retroviral overexpression of FAK yielded preB cells with comparable levels of FAK to empty vector-transduced proB cells. This

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Model of pro- and preB cell behavior. Left: IL-7 secreted by IL-7^{hi}CXCL12^{hi} stromal cells activates IL-7 receptor signaling in proB cells, which strengthens proB cell adhesion to IL-7^{hi}CXCL12^{hi} cells by increasing CXCR4 and α4β1 integrin activity. This positive feedback loop results in relatively stationary proB cells. PreBCR signaling breaks the cycle by further increasing CXCR4 activity and decreasing α4β1 integrin-mediated adhesion. As a result, preB cells are more motile and have lower exposure to IL-7. Changes in expression of focal adhesion kinase (FAK) between the pro- and preB cell stages are particularly prominent. Right: In the presence of preB cells with unrepaired double-stranded DNA (dsDNA) breaks, IL-7^{hi}CXCL12^{hi} cells decrease expression of IL-7 via an unknown mechanism.

resulted in increased phospho-STAT5 in preB cells, suggestive of increased IL-7 exposure, and a reduction in the ratio of preB cells to proB cells.

Lastly, [Fistonich et al. \(2018\)](#) asked whether B cells that had sustained DNA damage while rearranging their BCR positioned themselves differently from healthy developing B cells, as these cells' interactions with the niche may affect the likelihood of developing leukemia. Consistent with the possibility of a difference, RAG-mediated double-stranded DNA breaks induce a program in developing B cells that alters expression of genes that regulate migration ([Bredemeyer et al., 2008](#)). Although [Fistonich et al. \(2018\)](#) did not ob-

serve mislocalization of Artemis-deficient preB cells, they noticed that CXCL12^{hi}IL-7^{hi} cells (identified by *Cxcl12*-dsRed and *Il7*-GFP reporters) in the bone marrow of mice with Artemis-deficient preB cells expressed less *Il7*-GFP than in control animals. Similarly, mice that received preB acute lymphoblastic leukemia cells had lower *Il7*-GFP and *Cxcl12*-dsRed expression in the bone marrow than controls. These observations suggest the intriguing possibility that cells comprising the bone marrow niche limit the provision of survival factors to B cells that have improperly rearranged their BCR. Fascinating future directions will include defining what signals alert the niche to double-stranded DNA breaks in its occupants,

and determining whether there is a protective on-going process in which the microenvironment contributes to the removal of damaged B cells. This response may ultimately be coopted by leukemia, which in many instances alters the niche that supports normal hematopoiesis ([Schmidt et al., 2011](#); [Zhang et al., 2012](#); [Schepers et al., 2013](#); [Arranz et al., 2014](#); [Hanoun et al., 2014](#)). Understanding this interplay may suggest novel interventions in disease.

Acknowledgments

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