

authors did not test whether the lack of CalDAG-GEFI prevents activation of Cdc42, although the latter has other known GEFs. Instead, they suggest that activated Cdc42 somehow prevents CalDAG-GEFI from activating Rap-1. How this might occur is an intriguing topic for future study.

Although Artz et al do not address this possibility, signaling through TGF- $\beta$  receptors likely also dampens selectin-triggered integrin activation by inhibiting Rap-1. Engaging TGF- $\beta$  receptors may have other effects. For example, phosphatidylinositol-4-phosphate 5-kinase  $\gamma$  (PIP5KI $\gamma$ ) recruits talin-1 to membranes by direct binding and by generating phosphatidylinositol 4,5 bisphosphate (PIP2).<sup>3</sup> In lymphocytes, constitutively active Cdc42 decreases chemokine-induced generation of PIP2, implying that it negatively regulates PIP5KI $\gamma$ .<sup>5</sup> Thus, TGF- $\beta$ -receptor activation of Cdc42 could block talin-1 recruitment by inhibiting both Rap-1 and PIP5KI $\gamma$ . Artz et al also show that engaging TGF- $\beta$  receptors on monocytic cells inhibits chemokine activation of  $\alpha$ 4 integrins, at least in vitro. Therefore, TGF- $\beta$  receptors may modulate different integrins in different leukocyte subsets.

Artz et al demonstrate that TGF- $\beta$ 1 inhibits neutrophil integrin activation in vitro. Whether it exerts this function in vivo requires further investigation. Most cells express TGF- $\beta$ 1, which exerts pleiotropic effects depending on cellular context. These effects typically regulate gene expression, whereas Artz et al describe a rapid, transcription-independent signaling pathway. To become active, TGF- $\beta$ 1 must dissociate from a latency complex. Activated platelets release large amounts of latent TGF- $\beta$ 1 that can be activated by shear stress under some conditions.<sup>7</sup> Activated platelets bind to neutrophils arrested in venules and transmit signals that regulate integrin  $\alpha$ M $\beta$ 2-dependent crawling.<sup>8</sup> Could platelet-released TGF- $\beta$ 1 contribute to signaling?

Other molecules influence neutrophil integrins. Signaling through neutrophil adenosine A2A receptors hinders selectin- and chemokine-induced  $\beta$ 2 integrin activation, as well as  $\beta$ 2 integrin outside-in signaling.<sup>9</sup> On the other hand, rolling neutrophils secrete myeloid-related proteins 8 and 14 from the cytosol.<sup>10</sup> The secreted proteins bind in an autocrine manner to toll-like receptor 4 on neutrophils, triggering

Rap-1-dependent activation of  $\beta$ 2 integrins. The demonstration that TGF- $\beta$  receptors negatively regulate  $\beta$ 2 integrins further emphasizes the complexity of neutrophil responses during inflammation. Determining how diverse signaling pathways cooperate or compete in different contexts may suggest new therapies for inflammatory diseases.

*Conflict-of-interest disclosure: The author holds equity in Selexys and Tetherex Pharmaceuticals.* ■

## REFERENCES

1. Artz A, Butz S, Vestweber D. GDF-15 inhibits integrin activation and mouse neutrophil recruitment through the ALK-5/TGF- $\beta$ RII heterodimer. *Blood*. 2016;128(4):529-541.
2. Lefort CT, Ley K. Neutrophil arrest by LFA-1 activation. *Front Immunol*. 2012;3:157.
3. Calderwood DA, Campbell ID, Critchley DR. Talins and kindlins: partners in integrin-mediated adhesion. *Nat Rev Mol Cell Biol*. 2013;14(8):503-517.
4. Yago T, Petrich BG, Zhang N, et al. Blocking neutrophil integrin activation prevents ischemia-reperfusion injury. *J Exp Med*. 2015;212(8):1267-1281.

5. Bolomini-Vittori M, Montresor A, Giagulli C, et al. Regulation of conformer-specific activation of the integrin LFA-1 by a chemokine-triggered Rho signaling module. *Nat Immunol*. 2009;10(2):185-194.

6. Kempf T, Zarbock A, Widera C, et al. GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. *Nat Med*. 2011;17(5):581-588.

7. Ahamed J, Burg N, Yoshinaga K, Janczak CA, Rifkin DB, Collier BS. In vitro and in vivo evidence for shear-induced activation of latent transforming growth factor- $\beta$ 1. *Blood*. 2008;112(9):3650-3660.

8. Sreerankumar V, Adrover JM, Ballesteros I, et al. Neutrophils scan for activated platelets to initiate inflammation. *Science*. 2014;346(6214):1234-1238.

9. Yago T, Tsukamoto H, Liu Z, Wang Y, Thompson LF, McEver RP. Multi-inhibitory effects of A2A adenosine receptor signaling on neutrophil adhesion under flow. *J Immunol*. 2015;195(8):3880-3889.

10. Pruenster M, Kurz AR, Chung KJ, et al. Extracellular MRP8/14 is a regulator of  $\beta$ 2 integrin-dependent neutrophil slow rolling and adhesion. *Nat Commun*. 2015;6:6915.

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## ● ● ● LYMPHOID NEOPLASIA

Comment on Guo et al, page 553

# IL-4 regulates B-cell receptor signaling in CLL

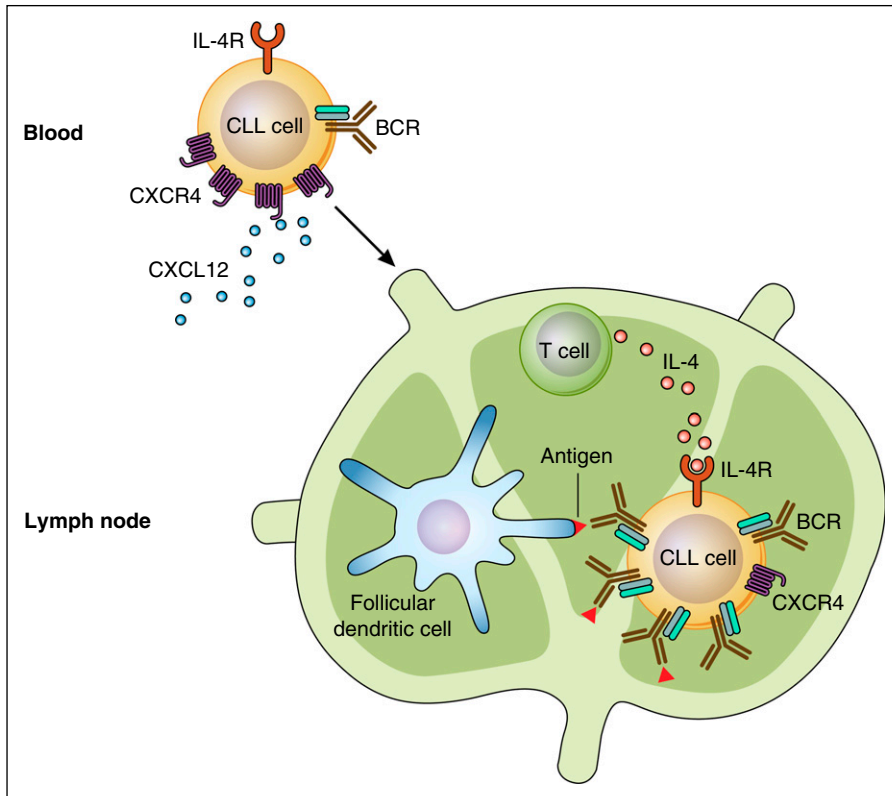
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In this issue of *Blood*, Guo et al show that interleukin-4 (IL-4) upregulates surface immunoglobulin M (IgM) expression and B-cell receptor (BCR) signaling capacity of chronic lymphocytic leukemia (CLL) cells, providing an explanation of how anergic CLL cells from the peripheral blood become signal competent when they enter the lymph nodes.<sup>1</sup>

The recent approval of the Bruton tyrosine kinase (BTK) inhibitor ibrutinib and the phosphatidylinositol 3-kinase  $\delta$  (PI3K $\delta$ ) inhibitor idelalisib represent major breakthroughs in the treatment of CLL. Although these drugs can inhibit various signaling pathways, they are believed to function primarily by inhibiting signals that the leukemic cells receive through their BCRs.<sup>2</sup> This mechanism of action is supported by numerous lines of evidence supporting a major role for the BCR pathway in the development and progression of CLL, including the expression of BCRs with particular structural and antigen-binding properties, the expression of high levels of

BCR-target genes in lymph node-derived CLL cells, and the significant association between clinical course and certain BCR-related features, such as the mutational status of the immunoglobulin heavy chain variable (IGHV) region genes.<sup>3</sup> This evidence, however, is difficult to reconcile with the relatively impaired capacity of CLL cells in the peripheral blood to respond to BCR engagement. Reduced BCR signaling capacity is a general feature of CLL, but is particularly prominent in the CLL subset with IGHV-mutated genes (M-CLL), where the malignant B cells are often completely anergic.<sup>4</sup>

In the article published in this issue of *Blood*, Guo et al investigate the mechanism(s)



Regulation of BCR expression on CLL cells by IL-4. CLL cells in peripheral blood have downmodulated BCRs, presumably to prevent inappropriate activation by antigen in the absence of co-stimulatory signals. In response to CXCL12 and other chemokines, these cells migrate to the lymph nodes, where locally secreted IL-4 induces CD79b and increases translocation of the BCR complex to the leukemic cell surface. This mechanism increases the likelihood that CLL cells encounter and interact with antigen in the appropriate context. IL-4R, IL-4 receptor. Professional illustration by Patrick Lane, ScEYence Studios.

responsible for the impaired BCR signaling capacity of freshly isolated peripheral blood CLL cells. They show that the reduced signaling capacity of the leukemic cells is at least in part caused by deficient expression of the CD79b component of the BCR, which is expressed at much lower levels in CLL than normal peripheral blood B cells. The relative deficiency of CD79b leads to inefficient assembly of the BCR complex and results in reduced translocation of the BCR to the leukemic cell surface. Importantly, Guo et al show that CD79b expression and BCR assembly can be rescued by IL-4, which is a cytokine that is abundantly produced by T cells of patients with CLL.<sup>5</sup> T cells interact with CLL cells in the lymph node proliferation centers,<sup>6</sup> suggesting that BCR expression and signaling capacity could be restored in CLL cells in lymph nodes by locally produced IL-4 (see figure).

Although the above described mechanism needs to be further validated by analyzing paired peripheral blood and lymph node CLL samples, it is supported by the observation of the authors that recently

egressed CLL cells express higher levels of surface IgM than CLL cells that have migrated from the lymph nodes into the peripheral blood at an earlier time point. A prominent feature of the latter cells are the high levels of the chemokine receptor CXCR4,<sup>7</sup> which drives the migration of CLL cells in response to the chemokine CXCL12. Interestingly, another study that was recently published in *Blood* reported that CXCR4 surface expression is downmodulated on CLL cells by IL-4.<sup>8</sup> This suggests that IL-4 could promote interaction of CLL cells with antigen by 2 mechanisms, ie, by upregulating CD79b and thus increasing surface IgM expression and BCR signaling capacity, and by downmodulating CXCR4 and promoting leukemic cell retention within the lymph node microenvironment.

Another important finding of the Guo et al study is the observation that IL-4 restores to a much greater extent surface IgM expression and BCR signaling capacity in the CLL subset with IGHV-unmutated genes (U-CLL), compared with M-CLL cells. Considering that the capacity to respond to stimulation with

external ligand is directly correlated with more aggressive disease both in human CLL<sup>9</sup> and in the E $\mu$ -TCL1 murine model,<sup>10</sup> this observation could provide additional insight into the mechanisms responsible for the more aggressive behavior of U-CLL than M-CLL. The reason for the greater capacity of U-CLL than M-CLL cells to upregulate CD79b in response to IL-4 is unclear at present and will require further study.

In conclusion, the study by Guo et al provides an explanation on how anergic CLL cells can be activated and induced to expand by antigen stimulation, a question that has puzzled CLL investigators for many years. The finding that surface IgM expression and BCR signaling capacity can be restored in CLL cells by IL-4 underscores the importance of signals from the microenvironment in determining the behavior of the leukemic cells, and further points to the BCR pathway as the primary target of BTK and PI3K $\delta$  inhibitors in CLL.

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## REFERENCES

- Guo B, Zhang L, Chiorazzi N, Rothstein TL. IL-4 rescues surface IgM expression in chronic lymphocytic leukemia. *Blood*. 2016;128(4):553-562.
- Wiestner A. The role of B-cell receptor inhibitors in the treatment of patients with chronic lymphocytic leukemia. *Haematologica*. 2015;100(12):1495-1507.
- Chiorazzi N, Efremov DG. Chronic lymphocytic leukemia: a tale of one or two signals? *Cell Res*. 2013;23(2):182-185.
- Mockridge CI, Potter KN, Wheatley I, Neville LA, Packham G, Stevenson FK. Reversible anergy of sIgM-mediated signaling in the two subsets of CLL defined by V<sub>H</sub>-gene mutational status. *Blood*. 2007;109(10):4424-4431.
- Rossmann ED, Lewin N, Jeddi-Tehrani M, Osterborg A, Mellstedt H. Intracellular T cell cytokines in patients with B cell chronic lymphocytic leukaemia (B-CLL). *Eur J Haematol*. 2002;68(5):299-306.
- Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. *Blood*. 2009;114(16):3367-3375.
- Calissano C, Damele RN, Marsilio S, et al. Intracloonal complexity in chronic lymphocytic leukemia: fractions enriched in recently born/divided and older/quiescent cells. *Mol Med*. 2011;17(11-12):1374-1382.
- Aguiar-Hernandez MM, Blunt MD, Dobson R, et al. IL-4 enhances expression and function of surface IgM in CLL cells. *Blood*. 2016;127(24):3015-3025.
- Le Roy C, Deglesne PA, Chevallier N, et al. The degree of BCR and NFAT activation predicts clinical outcomes in chronic lymphocytic leukemia. *Blood*. 2012;120(2):356-365.
- Iacovelli S, Hug E, Bannardo S, et al. Two types of BCR interactions are positively selected during leukemia development in the E $\mu$ -TCL1 transgenic mouse model of CLL. *Blood*. 2015;125(10):1578-1588.

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