stromal cells and endothelial cells. Neuronal stromal cell types, including mesenchymal is now thought to be anatomically located in stem cells. As AML in
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marrow stem and progenitor dysfunction. The most severely affected progenitor population is the megakaryocyte-erythroid-progenitor (MEP)—the progenitors responsible for the genesis of erythroid cells and platelets. In this study, Cheng et al do not address why MEPs are so drastically and selectively affected, leading to the bleeding complications so frequently observed in leukemias, but their model will doubtlessly be used to investigate this clinically important feature in the future.

Instead, Cheng et al focus on blood-forming stem cells. As AML infiltrates the marrow, they observed, hematopoietic stem cells (HSCs) became progressively quiescent and ceased cycling. Notably (and fortunately for patients), the quiescence is reversible: transplanted into nonleukemic hosts, these stem cells reacquired their functional capacity and resumed the genesis of mature cells.

Two broad mechanisms have been proposed to explain these effects of leukemia on normal stem cells. The first suggests that the effect occurs because AML cells “hijack” the normal microenvironments, or “niches,” of normal stem and progenitor cells, thereby leading to their dysfunction. Work from several groups has demonstrated that both normal and leukemia cells are dependent on specific stromal cells in the bone marrow for their function.1–7 For normal HSCs, the “niche” is now thought to be anatomically located in the perivascular region and is created by stromal cell types, including mesenchymal stromal cells and endothelial cells. Neuronal cells and bone-forming osteoprogenitors also regulate the physiology of HSCs.8

These microenvironmental interactions are known to maintain stem cell quiescence and can affect progenitor maturation. By disrupting these microenvironments, leukemia cells might cause a global disruption of stem and progenitor function. We might imagine this as a “broken windows/bad neighborhoods” theory: as AML cells infiltrate a perfectly viable “neighborhood” of a normal marrow, they disrupt the homes for stem and progenitor cells, and thereby cause the observed dysfunction of stem/progenitor cells.

A second theory posits that the AML cells directly alter the function of stem and progenitor cells. In this model, which we might call the “blood feud” theory, AML cells secrete factors, or interact directly with normal cells through cell-cell contacts, to cause their dysfunction. Cheng et al focused on this second theory. First, they demonstrate that as AML infiltrates the bone marrow, normal HSCs acquire progressive quiescence. What might explain the quiescence of HSCs in AML-infiltrated marrow? Bone marrow serum isolated from leukemia-infiltrated marrow was sufficient to enforce HSC quiescence, suggesting the presence of a soluble factor, or factors, that impinge on a signaling pathway in HSCs, restricting their proliferation.

To further elucidate this mechanism, Cheng et al flow-sorted HSCs from leukemia-infiltrated bone marrow and analyzed gene expression. As expected, cell cycle–related genes were profoundly downregulated. Notably, several genes were also upregulated. These included Hes-1 and a zinc finger–related transcription factor named Egr3. When HSCs from AML-occupied marrows were transplanted into normal recipients, the quiescence of the stem cells was relieved, and Egr3 levels returned to a lower baseline.

These experiments suggested that Egr3 might be a “strong limiting factor” that restricts the proliferation of HSCs. To determine if this was the case, Cheng et al knocked down Egr3 in HSCs, transplanted the cells into bone marrow, and then transplanted AML cells. In control HSCs (with intact Egr3), the previously observed quiescence was recapitulated. In Egr3 knockdown HSCs, in contrast, the AML infiltration was unable to induce HSC quiescence.

One notable feature of the experiment is that the effect of AML on hematopoietic stem cells, although mediated by Egr3, was still dependent on the “niche.” Although marrow–resident HSCs were rendered quiescent by AML, spleen–resident stem cells seemed to be unaffected by leukemic infiltration. The precise contributions of “niche-dependent” and “niche-independent” effects remains to be fully elucidated, but bad neighborhoods and bad residents clearly collaborate in altering hematopoiesis in leukemic patients. These observations serve as potent reminders that successful AML therapy may depend on not just killing leukemia cells in their niches, but also restoring the normalcy of bone marrow function.

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COMMENT ON WU ET AL

GVHD and miR: good things in small packages

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In this issue of Blood, Wu et al demonstrate that a cluster of microRNAs (miRs) previously shown to be important in malignant disease and B-cell development plays a critical role in the proinflammatory function of donor T cells that mediate acute graft-versus-host disease (GVHD).1

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There are increasing levels of intricacy in the fundamental processes of messenger RNA (mRNA) transcription and protein translation. One such intricacy involves the ability of double-stranded RNA to inhibit the generation of or mediate the degradation of mRNA. These noncoding RNAs are 18 to 24 nucleotides and form a double-stranded hairpin generated by the RNase III Dicer enzyme (see figure). miRs regulate protein synthesis by binding to the 3’ untranslated region to target specific mRNAs. This inhibition is mediated by recruitment of the RNA-induced silencing complex that mediates repression of protein translation and/or deadenylation and subsequent degradation of mRNA targets. miRs have an effect on more than 60% of all protein coding genes.

There are possibly more than 1000 different human miRs. One important miR is a polycistronic region found in humans on chromosome 13 that encodes 6 different miRs: miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92-1. This miR cluster is conserved among all vertebrates. Previous investigators indicated a role for miR-17-92 in the development of several malignant diseases. It is tightly linked to the function of E2F proteins, and the activity of the proapoptotic protein Bim. This cluster of miRs is critical to B-cell development. Several different functions have been found for this miR cluster in T cells, including regulation of effector and memory CD8+ T cells and Th1 T cells, and inhibition of regulatory T-cell responses. However, a role for the miR-17-92 cluster in the biology of GVHD has not been shown previously.

Wu et al used mice lacking the expression of the miR-17-92 cluster only in T cells to investigate the function of this miR cluster in the biology of acute GVHD. The authors observed that miR-17-92 was important for the proliferation, survival, and effector function of T cells. Donor T cells that lacked miR-17-92 mediated substantially diminished acute GVHD in 2 separate models: a complete mismatch model and a minor major histocompatibility complex mismatch model. Long-term donor T-cell reconstitution using knockout T cells came from wild-type bone marrow cells, which indicates the profound effect of loss of this miR cluster on donor T-cell function. Interestingly, these effects were associated with impaired accumulation of donor CD8+ T cells in the spleen and liver but there was no impact on donor CD8+ T cells at these sites. This was not expected because previous work suggested a clear function for miR-17-92 in the cytolytic and proliferative activity of CD8+ T cells. Acute GVHD is mediated predominantly by Th1 and Th17 cells. Wu et al found that the generation of donor Th1 and Th17 cells was markedly decreased in the gastrointestinal tract and mesenteric lymph nodes after the transplantation of T cells that were unable to generate miR-17-92. However, there was an increase in donor cells in the spleen that generated interleukin-4 (IL-4) and IL-5 consistent with a Th2 phenotype. Thus, one important function of the miR-17-92 cluster is polarizing T cells toward a proinflammatory Th1/Th17 profile and away from the generation of Th2 cells after allogeneic transplant.

To be clinically relevant, approaches that diminish GVHD cannot have a large impact on the graft-versus-tumor effect. Wu et al used 2 different tumor models to demonstrate that T cells lacking miR-17-92 were still functional in eliminating tumor cells. This may have been the result of the relative preservation of the cytolytic function of donor CD8+ T cells in the absence of miR-17-92. One criticism of genetic approaches in preclinical models is the ability to target a specific pathway only in donor T cells, which would not be possible using clinical approaches. To circumvent this
concern, Wu et al made use of locked nucleic acid (LNA) antagonists targeting miR-17-92. An LNA is an RNA molecule in which an additional methylene bridge fixes the conformation of the ribose moiety. Antagomirs are inhibitors of miRs that are altered to improve their resistance to degradation. Wu et al used 2 different antagonists that targeted either the miR-17 or miR-19 seed family. The miR-19 antagonist was more effective than the miR-17 in decreasing interferon γ production by CD4+ and CD8+ T cells and improving survival (see figure). Thus, the use of the miR-19 antagonist represents a novel approach to prevent acute GVHD.

One question not addressed by the Wu et al study is the mechanism of action of miR-17-92 in the biology of acute GVHD. There are more than 5000 mRNA targets for miR-19a alone, and previous studies indicate that this miR cluster targets PTEN, TGFBR1, SMAD2, and SMAD4 as well as BCL2L11 (Bim), E2F2, and E2F3. If miR-17-92 is critical to diminishing the function of the transforming growth factor β (TGF-β) pathway, blocking its function could conceivably diminish GVHD via the generation of tolerogenic antigen-presenting cells, via the effects of TGF-β on the migration of immune cells, or perhaps through the expansion of inducible regulatory T cells. This will need to be investigated in future studies.

In terms of future approaches in transplantation biology, this cluster is critical for the generation of pre-B cells. Thus, it is important to determine whether blocking the function of miR-17-92 would have an impact on B-cell homeostasis and/or chronic GVHD. Are the antagomirs of miR-19 able to treat acute and/or chronic GVHD? Is this activity altered in the presence of calcineurin inhibitors and/or steroids? These and other questions will be important as these approaches move closer to use in the clinic.

The Wu et al1 study adds to a small but growing list of publications implicating miRs in the biology of acute GVHD.6,7 A previous publication demonstrated an increase in the plasma for specific miRs in patients with acute GVHD that included miR-423, miR-199a-3p, miR-93*, miR-377, miR-155, and miR-30a but, interestingly, not this miR cluster. It is anticipated that long noncoding RNA species will be shown to have an impact on the biology of acute GVHD in the not too distant future. These new revelations show that a complex disease is becoming more complex, but this complexity may offer clues to future therapy.

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**Comment on Lafouresse et al, page 1336**

**Directing CLL-cell traffic**

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In this issue of *Blood*, Lafouresse et al visualized the in vivo behavior of human chronic lymphocytic leukemia (CLL) cells within the mouse lymph node (LN) microcirculation and discovered that CLL cells bind to LN high endothelial venules (HEVs) via an L-selectin (CD62L)-dependent adhesion process. They also found that CLL cells from patients treated with the B-cell receptor (BCR) inhibitor idelalisib decrease their levels of L-selectin and reduce binding to LN HEVs.

HEVs are specialized blood vessels that allow the entry of normal B lymphocytes into LN-specific microenvironments. Normal B cells bind to HEVs through a multistep adhesion cascade process that entails rolling, sticking, and crawling. The mechanisms that regulate CLL-cell migration in vivo remain incompletely understood even though it is known that chemokines and chemokine receptors play a central role in malignant B-cell traffic. As the microenvironment exerts a critical role in CLL development and progression, it is important to determine which elements direct CLL-cell trafficking and to identify which molecules control the initial capture of CLL cells by HEV walls, allowing their entry and re-entry in LNs. Understanding this mechanism would have important implications. First, it might help in defining the molecular features that account for the clinical presentation of small lymphocytic lymphoma (SLL), which differs from CLL because of the presence of bulky LNs with only minimal-to-moderate peripheral blood lymphocytosis. Second, it may shed some light onto the mechanisms that are at the basis of the profound CLL-cell redistribution observed in patients treated with BCR inhibitors such as the phosphoinositide 3-kinase δ inhibitor idelalisib.

By intravital microscopy and multiphoton imaging in vivo imaging (see figure), the authors investigated the mechanisms that allow CLL-cell entry and/or re-entry in LNs. They observed that CLL cells from patients with bulky disease and elevated numbers of circulating lymphocytes endowed with high levels of L-selectin have a significantly increased rolling fraction and decreased rolling velocity. Conceivably, the decreased rolling velocity facilitates a chemokine-induced integrin-mediated arrest of CLL cells. Along this line an increased rolling velocity was evident in cells expressing lower levels of L-selectin obtained from CLL patients without bulky disease and a reduced rolling
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Jonathan Serody