1. Hsu C, MacGlashan D Jr. IgE antibody up-regulates high affinity IgE binding on murine bone marrow-derived mast cells. Immunol Lett. 1996; 52:129-134.

2. Yamaguchi M, Lantz CS, Oettgen HC, et al. IgE enhances mouse mast cell FcεRI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. J Exp Med. 1997;185:663-672.

3. Kalesnikoff J, Huber M, Lam V, et al. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. Immunity. 2001;14:801-811.

4. Asai K, Kitaura J, Kawakami Y, et al. Regulation of mast cell survival by IgE. Immunity. 2001;14: 791-800.

5. Kitaura J, Song J, Tsai M, et al. Evidence that IgE molecules mediate a spectrum of effects on mast cell survival and activation via aggregation of the FcεRI. Proc Natl Acad Sci U S A. 2003:100: 12911-12916.

6. James LC, Roversi P, Tawfik DS. Antibody multi-specificity mediated by conformational diversity. Science. 2003;299:1362-1367.

Bridging the bone marrow–thymus gap

Most T cells are generated in the thymus. Although adult thymi contain highly proliferating progenitor cells, these are not self-renewing and only produce mature T cells over several weeks. Thus, continuous thymic T-cell output is dependent on new thymic immigrants. All T cells are derived from hematopoietic stem cells (HSCs) that reside in the bone marrow and, occasionally, travel through blood.[1] If thymus entry is exclusive, it could be HSCs themselves or defined downstream T-cell progenitors that immigrate. Alternatively, multiple circulating cells enter the thymus and only the ones that can respond to thymic T-cell differentiation signals will proliferate. When and where does T-lineage commitment occur, and what are the characteristics of physiologic thymus-seeding cells? Answering these questions will help improve T-cell generation when urgently needed.

Differentiation of HSCs to mature hematopoietic cells involves loss of self-renewal potential followed by restriction of developmental options (ie, the gradual commitment to different hemato-lymphoid lineages). Using flow cytometry, viable cell populations can be purified according to their expression of biologically relevant markers. It has been shown that bone marrow contains single cells that harbor exclusively lymphoid or myeloid developmental potential (common lymphoid progenitors [CLPs] and common myeloid progenitors [CMPs]). Mouse CLPs and CMPs are capable of protecting animals from lethal infections that are primarily controlled by the lymphoid or myeloid arm of the immune system, respectively. These findings prove a lympho-myeloid dichotomy in bone marrow that results in functionally relevant progenitor cells.[1] Thus, interleukin-7 receptor α–positive (IL-7Rα+) CLPs or their progeny were strong candidates for being thymus-seeding cells. Indeed, pre-T α–expressing CLP progeny in bone marrow, termed CLP-2, were shown to be capable of immigrating to the thymus and producing T cells.[2] However, the concept that CLPs are the major physiologic T-cell progenitors was challenged by experiments where HSCs, CLPs, and thymus-derived progenitors (ETPs) were directly compared: ETPs were capable of generating over a longer time period more thymocyte progeny than CLPs in vivo, and, in contrast to CLPs, had some myeloid potential.[3] This implies that ETPs arise from HSCs without proceeding through a CLP state. Do circulating HSCs themselves then enter the thymus, immediately lose self-renewal potential, and rapidly commit to ETPs upon environmental cues such as Notch ligands? It has been argued that this is not the case because intrathymically injected HSCs that were isolated again from thymi after 3 days maintained at least short-term self-renewing potential, and robust lympho-myeloid repopulation potential in secondary transplantations, a capacity not found in normal thymi.[4]

In an extension of their previous work,[5] Perry and colleagues (page 2990) now add important information to bridge the bone marrow–thymus gap. They identified candidate immediate precursors of thymus-seeding cells in bone marrow that are functional and phenotypic counterparts of thymic ETPs. As ETPs, they express L-selectin (which might be of importance although not essential in thymic homing), are mostly IL-7Rα−, and produce upon intravenous transfer major waves of thymic T cells, but minor B-cell and very low myeloid engraft-ment, and do not rescue lethally irradiated recipients. In terms of phenotype and function, L-selectin+ bone marrow progenitors overlap and likely contain the recently defined earliest Rag+ lymphocyte progenitors (ELPs)[6] that are supposed to be upstream of CLPs and ETPs.[3]

Although the existence of common lymphoid- or myeloid-restricted progenitors is not questioned, these new data add to growing evidence that during successive lineage commitment, progenitors are generated that incline to a lineage but maintain alternative options that could become relevant upon changing environmental stimuli. In this view, the migration of progenitor cells will greatly influence their contribution to any given mature cell. This is not reflected well by in vitro or in vivo transfer experiments. Most of the irreversible T-cell commitment might occur in the thymus. However, marking of thymus-seeding progenitors that initiate a proliferative T-cell burst and ultimate tracing of the steady-state marrow-blood-thymus sequence still need to be accomplished.

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1. Kondo M, Wagers AJ, Manz MG, et al. Biology of hematopoietic stem cells and progenitors: implications for clinical application. Annu Rev Immunol. 2003;21:759-806.

2. Martin CH, Aifantis I, Scimone ML, et al. Efficient thymic immigration of B220+ lymphoid-restricted bone marrow cells with T precursor potential. Nat Immunol. 2003;4:866-873.

3. Allman D, Sambandam A, Kim S, et al. Thymopoiesis independent of common lymphoid progenitors. Nat Immunol. 2003;4:168-174.

4. Mori S, Shortman K, Wu L. Characterization of thymus-seeding precursor cells from mouse bone marrow. Blood. 2001;98:696-704.

5. Perry SS, Pierce LJ, Slayton WB, Spangrude GJ. Characterization of thymic progenitors in adult mouse bone marrow. J Immunol. 2003;170:1877-1886.

6. Igarashi H, Gregory SC, Yokota T, Sakaguchi N, Kincade PW. Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. Immunity. 2002;17:117-130.

"Annexing" acute leukemias

A hemorrhagic disorder associated with enhanced thrombin activation and disseminated intravascular coagulation is often observed in acute promyelocytic leukemias

(APLs) carrying the t(15;17). In these leukemias high levels of annexin II, a calcium-regulated and phospholipid-binding cell surface protein, correlate with increased propensity to hemorrhage. Treatment with all-*trans*-retinoic acid (ATRA) resolves the coagulapathy, and is associated with a concomitant down-regulation of *annexin II* transcript and protein levels. But what of the role of annexin II in the pathogenesis of hemorrhagic disorders associated with leukemia?

Annexin II is thought to have a thromboregulatory role by enhancing the tissue plasminogen activator–dependent formation of plasmin on endothelial cell surfaces. Annexin II overexpression on the surface of APLs may in fact lead to uncontrolled production of plasmin, shifting the hemostatic balance toward overt bleeding.[1] A definitive
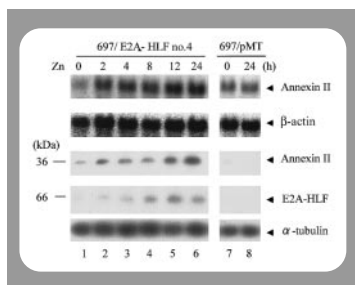
role for annexin II in maintaining fibrin homeostasis and plasmin regulation has been demonstrated in annexin II null mice, providing a dramatic link to coagulopathy.[2]

In this issue of *Blood*, Matsunaga and colleagues (page 3185) report that annexin II is also expressed at high levels in each of 4 t(17;19) acute lymphoid leukemic (ALL) cell lines. The t(17;19) encodes the uncommon but well-studied chimeric oncoprotein E2A-HLF, a basic-region leucine zipper (bZIP) DNA-binding protein that contains the heterologous E2A transactivation domains. Patients with E2A-HLF–induced leukemias are refractory to conventional chemotherapeutic treatment and have a generally poor prognosis that is associated with hypercalcemia and hemorrhagic complications. E2A-HLF has been implicated to transform B-cell progenitors by several potential pathways, including enhanced sur-

vival (through induction of SLUG, a transcriptional repressor that shares homology with the *Caenorhabditis elegans* apoptotic repressor Ces1) and impaired differentiation.[3-4] Interestingly, genetic studies have also implicated annexin VIII, another member of the annexin family, as a potential downstream gene, suggesting a possible mechanistic basis for the leukemia-associated bleeding disorder.[5]

Matsunaga et al now provide experimental evidence that annexin II is a downstream target of E2A-HLF and that in IL-3–dependent cells annexin II expression is regulated by IL-3 and Ras pathways. Moreover, E2A-HLF expression in these cells induced annexin II expression in the absence of IL-3, indicating that E2A-HLF induces annexin II by substituting for cytokines that activate downstream pathways of Ras. They noted that annexin II expression was unlikely to contribute to the cell survival pathways that E2A-HLF trigger since conditional expression of annexin II was unable to stem cytokine deprivation–induced apoptosis. Matsunaga and colleagues asked this important question: what role, if any, does annexin II play in E2A-HLF–induced ALL coagulopathy? They noted that while total annexin II protein levels were increased in 4 E2A-HLF–bearing cell lines tested, the cell surface levels were fairly divergent and in one example did not correlate with patient coagulopathy. More likely, they report, surface annexin II could be correlated with hypercalcemia at onset, the other rare complication in pro-B ALL. One other intriguing aspect is that annexin II interacts with procathepsin B on the surface of tumor cells, and is involved in extracellular proteolysis, facilitating tumor invasion and metastasis. E2A-HLF–positive leukemia is characterized by bone invasion and hypercalcemia, both paraneoplastic syndromes that are rare complications in other types of childhood acute B-lineage leukemia, offering yet another possible clue. Certainly future studies will shed light on the role annexin II expression plays in these rare ALLs.

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1. Menell JS, Cesarman GM, Jacovina AT, et al. Annexin II and bleeding in acute promyelocytic leukemia. N Engl J Med. 1999;340:994-1004.
2. Ling Q, Jacovina AT, Deora A, et al. Annexin II regulates fibrin homeostasis and neoangiogenesis in vivo. J Clin Invest. 2004;113:38-48.
3. Inukai T, Inoue A, Kurosawa H, et al. SLUG, a ces-1-related zinc finger transcription factor gene with antiapoptotic activity, is a downstream target of the E2A-HLF oncoprotein. Mol Cell. 1999;4: 343-352.
4. Smith KS, Rhee JW, Cleary ML. Transformation of bone marrow B-cell progenitors by E2a-Hlf requires coexpression of Bcl-2. Mol Cell Biol. 2002; 22:7678-7687.
5. Kurosawa H, Goi K, Inukai T, et al. Two candidate downstream target genes for E2A-HLF. Blood. 1999;93:321-332.

MLL, *Hox* genes, and leukemia: the plot thickens

The mixed lineage leukemia gene *MLL*, a histone methyltransferase and the human homolog of *Drosophila* trithorax, is rearranged in a variety of acute lymphoid and myeloid leukemias. Over the past 2 years great progress has been made in understanding the mechanism by which MLL fusion proteins transform. MLL binds to promoters (and probably other sequences) of *Hox* genes such as *Hoxa7* and *Hoxa9* to maintain their expression.[1,2] These Hox proteins regulate hematopoiesis and are normally expressed only in early hematopoietic progenitors. MLL fusion proteins also directly up-regulate *Hox* expression but in contrast to the wild-type MLL do not allow for the normal down-regulation of *Hox* expression.[1,3,4] This persistent expression of *Hox* genes along with expression of another up-regulated Hox cofactor, Meis1, appears to be necessary and sufficient to cause leukemia.[4] This is supported by previous work from the Cleary laboratory that showed that MLL-ENL (eleven-nineteen leukemia) could not transform *Hoxa9* knockout bone marrow.[5] Furthermore, using a conditionally transforming version of MLL-ENL, collaborator Robert Slany's laboratory and mine found that expression of *Hoxa9* plus *Meis1* was sufficient to completely replace the gain of function activity of MLL-ENL.[4]

It was all beginning to look pretty simple—that is until the paper by So and