(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 coagulopathy, 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. 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.

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 unligated 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 survival (through induction of SLUG, a transcriptional repressor that shares homology with the Caenorhabditis elegans apoptotic repressor Ces1) and impaired differentiation. 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.

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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**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. 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. 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. 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. 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. It was all beginning to look pretty simple—that is until the paper by So and
colleagues (page 3192) in this issue of Blood. The authors found that, in contrast to MLL-ENL, another MLL fusion protein, MLL-GAS7, was able to transform Hoxa7 or Hoxa9 knockout bone marrow. One possible explanation is that the 2 fusion proteins have intrinsically different mechanisms of action. MLL-ENL, a commonly occurring translocation, fuses MLL to a potent transcriptional transactivator. MLL-GAS7 appears to transform through dimerization of the truncated MLL molecule. While both MLL-ENL and MLL-GAS7 up-regulate Hox genes, it is possible that the dimerized form of MLL activates additional targets. In the study by Zeisig et al, MLL fusion proteins up-regulated a number of putative oncoproteins outside the Hox genes, such as Lmo2, Flt3, and N-Myc, that possibly contribute to transformation. The findings of So et al do not appear to be unique to MLL fusion proteins that transform by dimerization because studies of Mll-AF9 knock-in mice from John Kersey’s laboratory show that this fusion protein, which is very similar to ENL, also develops leukemia in the absence of functional Hoxa9. What to make of all this? Perhaps the most likely explanation is that Hox genes still are the critical targets; however, MLL fusion proteins up-regulate multiple Hox genes (including Hoxa5, Hoxa7, Hoxa9, Hoxa10, and Meis1 among others), and under the right conditions loss of any individual Hox protein can be compensated for by expression of the others. This work raises serious questions about whether developing therapy directed against any individual Hox protein would be successful. Using powerful tools like knockout mice and RNAi, it should not be long until we know the answer.

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Novel twin study implicates IL-6 in the etiology of young adult Hodgkin lymphoma

Cytokines are small secreted or membrane-bound proteins that act in both an autocrine and paracrine fashion and have a central role in the development and regulation of the immune system. There is increasing interest in the role of these proteins in cancer etiology and pathogenesis. Interleukin 6 (IL-6) is a pro-inflammatory, pleiotropic cytokine produced by multiple cell types, including normal and malignant B and T lymphocytes. Growing laboratory and clinical evidence supports the hypothesis that IL-6 plays a role in the pathogenesis of hematologic and other malignancies. In Hodgkin lymphoma specifically, IL-6 levels are elevated in untreated and relapsed patients and in patients with a poorer prognosis. However, it has not been shown to date whether higher IL-6 levels before diagnosis (and thus unaffected by disease or treatment) are associated with risk of developing Hodgkin lymphoma, which would support an etiologic role for IL-6.

The article by Cozen and colleagues (page 3216) that appears in this issue of Blood addresses the association of IL-6 with the development of young-onset Hodgkin lymphoma (diagnosed under age 50 years) using a novel study design that integrates both genetic and phenotypic data. Using a twin registry, the authors recruited 88 young adult Hodgkin lymphoma patients, their twins, and unrelated age-matched controls, creating triads. Peripheral blood was collected on the same day from each member of the triad, shipped, and processed together. The unaffected twin of monozygotic twin pairs was used as a “surrogate case” whose immune characteristics reflect those of the affected twin, at least to the extent that IL-6 levels are genetically controlled but are not influenced by Hodgkin lymphoma or its treatment. Cozen et al found that unaffected monozygotic twins of the Hodgkin lymphoma cases had IL-6 levels that were approximately 70% greater compared with controls. The IL-6 gene also has a functional single nucleotide polymorphism (SNP) in the promoter region (−174G>C), such that the CC genotype is associated with lower levels of IL-6. In their study, affected twins were 70% less likely to have the low-risk (CC) genotype compared with controls, and there was a dose response with the number of low-risk (C) alleles. Finally, among unaffected twins and controls there was an inverse association of IL-6 levels with the number of C alleles, linking phenotype with genotype in this study population.

Taken together, these provocative results support the hypothesis that high levels of genetically determined IL-6 are associated with risk of young adult Hodgkin lymphoma. Is this a causal association? Maybe, but it is too early to make this conclusion. Replication of these findings will be needed. It must also be kept in mind that IL-6 is unlikely to be a lone player, as it is a part...
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