Comment on Chen et al, page 2036

More ZAP for chronic lymphocytic leukemia (CLL)

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Antigen stimulation of the leukemic clone is increasingly implicated in the pathogenesis of CLL. Chen and colleagues provide evidence that expression of ZAP-70 in CLL B cells renders IgM signaling more effective and thereby could contribute to the more rapidly progressive clinical course of ZAP-70–positive CLL.

There is increasing evidence for a role of antigen in the pathogenesis of chronic lymphocytic leukemia (CLL), most notably the nonrandom composition of the B-cell receptor (BCR) expressed on the leukemic cells, suggesting that CLL B cells recognize distinct antigens. Ongoing antigen-mediated activation of the leukemic cells through the B-cell receptor is suggested by the presence of cellular activation markers on the cell surface and the expression of genes induced by BCR stimulation in at least some CLL types.

Other features such as the low expression of the immunoglobulin chains on the cell surface and reduced responsiveness to immunoglobulin M (IgM) stimulation in some CLL clones are consistent with an anergic state induced by chronic antigen exposure. Importantly, the nature of the cognate antigen and differences in the responsiveness of the leukemic cells to antigen stimulation may explain the well-known heterogeneity in the clinical course of CLL.

Zeta-associated protein of 70 kDa (ZAP-70), a cytoplasmic tyrosine kinase essential for T-cell–receptor signal transduction, is preferentially expressed in CLL B cells whose immunoglobulin genes have not undergone somatic hypermutation (Ig-unmutated CLL), while a second CLL subtype with mutated immunoglobulin genes most often lacks ZAP-70 expression. Expression of ZAP-70 in CLL B cells is associated with more rapid disease progression and shorter survival and may be a better predictor of clinical course than Ig-mutation status. Whether ZAP-70 expression indicates a distinct cellular origin of the clone or different activation states of the leukemic cells remains to be determined. Chen and colleagues focused on a role of ZAP-70 in BCR signal transduction and demonstrated that CLL B cells that express ZAP-70 are more likely to respond to IgM cross-linking with increased tyrosine phosphorylation of key signal transduction proteins and increased calcium flux than ZAP-70–negative CLL B cells. By introducing ZAP-70 into ZAP-70–negative CLL cells, they could convert IgM-nonresponsive B cells to IgM-responsive cells. While this finding supports a direct role of ZAP-70 in IgM signaling, it is not evident how expression of ZAP-70 increases BCR signaling in CLL B cells, as these cells do express normal amounts of SYK kinase, the functional B-cell homolog of ZAP-70. It is conceivable, however, that ZAP-70 expression may antagonize mechanisms involved in downmodulating BCR activity in anergic B cells and thereby could facilitate BCR signaling.

The evidence implicating ZAP-70 in BCR signaling is derived from in vitro experiments using a controlled but artificial way to initiate IgM signaling. It is therefore important to test predictions of this model in vivo and to keep in mind that ZAP-70 expression distinguishes 2 CLL subtypes that differ in several respects, not the least of which may be the nature of the antigen recognized and possible differences in the interaction with nonmalignant cells and in BCR composition. All of these factors may influence the consequences of BCR engagement in vivo that can range from increased proliferation and survival to induction of apoptosis.
Can we afford to let sleeping dogs lie?

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In patients with chronic myelogenous leukemia (CML) mutations of the BCR-ABL kinase domain (KD) have been identified as the leading cause of acquired resistance to imatinib, while the mechanisms underlying the persistence of minimal residual disease (MRD) are unknown. In this issue of Blood, Chu and colleagues report several patients with KD mutations at the time of complete cytogenetic response to imatinib. See the complete figure in the article beginning on page 2093.

Imatinib induces complete cytogenetic response (CCR) in most patients with chronic myelogenous leukemia (CML), but minimal residual disease (MRD) remains detectable by reverse transcriptase–polymerase chain reaction (RT-PCR) in all but a few cases. This is not just a cosmetic problem, as anecdotal observations reported rapid disease recurrence after discontinuation of imatinib, an indication that the residual BCR-ABL–positive cells retain full leukemogenic potential.1,2 Furthermore, patients who receive imatinib as primary therapy for CML may progress to blast crisis directly from CCR.3

Reactivation of BCR-ABL kinase activity is common in patients who relapse after an initial response to imatinib. Most of these individuals harbor mutations in the kinase domain (KD) of BCR-ABL that impair drug binding.4 In contrast, the mechanisms responsible for persistence of MRD in responding patients are not well understood. Chu and colleagues have studied CD34+ cells from patients with CCR for KD mutations. They found mutations in 5 of 13 patients at the initial evaluation, and in 4 additional patients at follow-up, when rising BCR-ABL mRNA levels were detected by quantitative RT-PCR, while CCR was still maintained. Intriguingly, most of the mutations in these patients conferred only moderate resistance to imatinib in proliferation and phosphorylation assays, suggesting they may be capable of preventing the extinction of the leukemic clone but are barely able to support its expansion. Thus, KD mutations may be responsible for disease persistence in a subset of patients with CCR. A few issues, however, are curious. Although the frequency of mutations has not been studied systematically in patients with CCR, the incidence reported by Chu et al appears to be high compared with an unselected cohort of CML patients on imatinib.5 One explanation for this discrepancy may be that the group under study may be high risk, consistent with the fact that the rate of overt relapse or rising levels of BCR-ABL mRNA on follow-up was certainly higher than one would expect in standard risk patients with CCR. Thus, these patients may have been caught on their path to disease progression rather than in stable remission. The other possibility is that the technique used—amplification of Bcr-Abl from CD34+ cells and sequencing of multiple individual clones—may be instrumental for detecting mutant clones in CCR patients. Whether this approach would detect KD mutations at an appreciable frequency in patients with stable MRD must be addressed in future studies. Another intriguing observation is that mutants such as Y353H are equally or even more sensitive to imatinib than wild-type BCR-ABL but nonetheless grow out over time, suggesting that they may increase the transforming potency of BCR-ABL irrespective of imatinib or that another resistance mechanism may be present. From a therapeutic standpoint, it would be good news if KD mutants were found to cause disease persistence since they would be targets for alternative Abl kinase inhibitors.

Which other mechanisms may underlie disease persistence? Quiescent BCR-ABL–positive progenitor cells are present in CML patients that are capable of repopulating severe combined immunodeficient (SCID) mice. Treated with imatinib ex vivo, these cells survive drug concentrations that are lethal to proliferating CML progenitor cells.6 There is

Sensitivity of TF-1 cells expressing KD mutants isolated from CML patients at the time of complete cytogenetic response to imatinib. See the complete figure in the article beginning on page 2093.
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