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occurred in one-quarter of patients, with responses being more common in patients with CLL/SLL (54.5%) and DLBCL (23.5%), and less common in follicular lymphoma (9.5%) or mantle cell lymphoma (11.1%). Evidence of clinical activity was also noted in a number of other patients, although it was insufficient to qualify for a response. The median progression-free survival was 4.1 months for all patients.

Other than CLL/SLL, the response rates with fostamatinib were modest. However, single-agent activity is not essential for pursuing a novel targeted agent. The role of such drugs may primarily be to enhance the efficacy of other agents. Perhaps the poster child for this effect is bevacizumab (Avastin; Genentech Biooncology). Recent data suggest that, at least in CLL, the combination of R406 with fludarabine increases cytotoxicity compared with fludarabine alone. Moreover, in B-CLL cells, the cytotoxic effects of Syk correlates with Syk expression.[3]

B-cell diseases are markedly heterogeneous, even within histologic subtypes, as has been repeatedly demonstrated by gene-expression profiling and other technologies.[4] Thus, differences in response may reflect variability in the inherent biology of a specific tumor. The goal is to individualize lymphoma therapeutic strategies based on molecular/genetic features of the tumor and pharmacogenomic characteristics of the patient. Therefore, it is critical to include correlative studies within clinical trials to demonstrate whether the relevant target is actually affected. Future strategies based less on nonspecific cytotoxics and more on combinations of targeted therapies directed at different receptors and pathways will bring us closer to limiting untoward effects while enhancing efficacy, resulting in cure of patients with B-cell malignancies.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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Comment on Mansson et al, page 2601

There's many a CLP on the path to B

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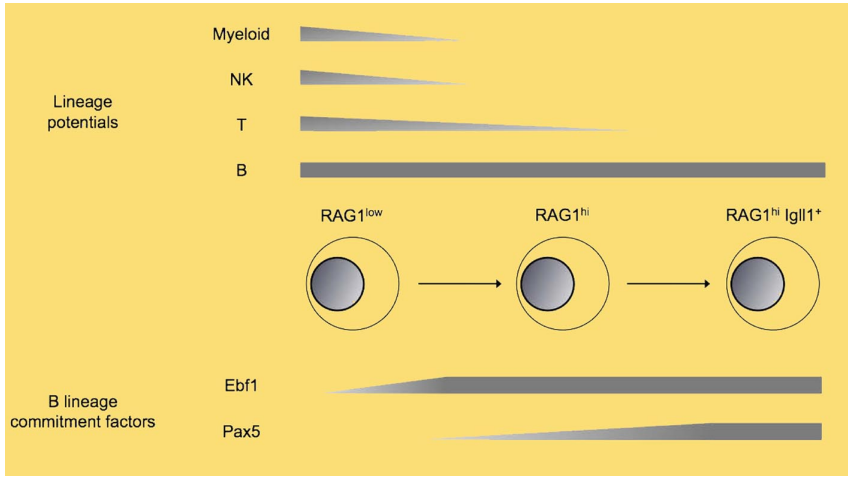
Lymphocyte development is an excellent model system to study cell fate choices and the underlying transcriptional mechanisms. However, the earliest events have been poorly understood, due in part to the rarity of the relevant cellular intermediates. In this issue of *Blood*, Mansson and colleagues identify several subsets within the CLP and fraction A population ($B220^+$ CLP phenotype) with differing lineage potentials.[1] The results indicate that B-lineage restriction initiates at much earlier developmental stages than previously thought and provide insight into the hierarchical transcriptional mechanisms involved in B-lineage commitment.

How common lymphoid progenitors (CLPs) give rise to B lineage–restricted progeny is unclear. The B lineage–specific transcription factor Pax5 is critical for B-cell development, and it was suggested that expression of Pax5 as visualized by up-regulation of its gene target CD19 on progenitors drove B-cell lineage commitment.[2,3] However, $CD19^-$ CLPs express transcriptional regulators important for B-cell lineage commitment including Ebf1 and Pax5, but surprisingly still maintain non–B-cell lineage potentials.[3,4] Mansson et al used mice transgenic for reporters of RAG1 and Igll1 (itself a transcriptional target of Pax5) to identify substantial heterogeneity within the $CD19^-$ CLP/fraction A pool. The authors identified in this early progenitor compartment some cells that were $RAG1^{low}$, other cells that were $RAG1^{hi}$ but negative for Igll1 (referred to here as $RAG1^{hi}$), and finally cells that were $RAG1^{hi}$ and also positive for Igll1 (referred to here as $Igll1^+$). In vitro cell culture established that these newly identified populations were linearly related, with a developmental sequence of $RAG1^{low}$ to $RAG1^{hi}$ to $Igll1^+$.

Consistent with this proposed relationship, the earliest $RAG1^{low}$ cells had the broadest set of lineage potentials, maintaining natural killer (NK), B, T, and even a degree of myeloid potential in clonal assays. The potential to generate NK cells and also myeloid cells appeared greatly decreased by the $RAG1^{hi}$ stage (see figure). This finding is supported by other recent data showing that RAG1 expression in CLP marks diminished NK-cell potential at the population level.[5] Expression of the cell-surface marker Ly6D correlated with increased expression of the RAG1 reporter and also coincided with loss of NK-cell potential. Other recent work has found that the $Ly6D^+$



Subsets within the bone marrow CLP/fraction A pool with differing lineage potentials and expression of B-lineage transcriptional regulators.

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pool of CLP does not possess efficient T-lineage potential in vivo.[6] However, the Ly6D$^+$ subset of CLP did possess a degree of T-cell potential when assessed in vitro in this study.

This new work suggests a novel hypothesis regarding the molecular mechanisms that underpin B-cell lineage commitment. The authors found that the bulk of NK-cell potential is lost in RAG1^{hi} cells, where levels of the early B-cell specification factor Ebf1 are high, but Pax5 is relatively low (see figure). This could suggest that NK potential is attenuated through an Ebf1-dependent but Pax5-independent mechanism. It is also interesting to note that the majority of myeloid potential appears lost by the RAG1^{hi} stage, supporting recent work that proposes Ebf1 may also be involved in suppressing myeloid potential of developing B-cell progenitors in a Pax5-independent manner.[7] However, T-lineage potential is not lost until the stage where the Pax5-dependent target Igll1 is up-regulated, suggesting that final commitment to the B-cell lineage does require higher levels of Pax5 expression. Further insights may develop from examination of Ebf1- and Pax5-deficient progenitors at these newly defined stages of early B-cell development.

The identification of these populations contained within the CLP/fraction A pool clarifies some confusing aspects of early lymphocyte development. The new work will also allow us to better study the molecular mechanisms underlying B-cell lineage commitment. The demonstration that Ly6D is up-regulated at these earliest described steps of B-lineage restriction allows these early transitions to be traced in normal mice, without the requirement for elegant but onerous analysis of multiple transgenic reporter strains. In a difficult and sometimes perplexing area, this paper together with the other recent work discussed here represents significant progress.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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Comment on Lapalombella et al, page 2619

The 3 Rs in CLL immune dysfunction

Alan G. Ramsay and John G. Gribben BARTS AND THE LONDON SCHOOL OF MEDICINE

In this issue of *Blood*, Lapalombella and colleagues present evidence that the immunomodulatory drug lenalidomide (Revlimid) may repair and reverse the humeral defect in CLL.[1]

Clinical response rates have improved significantly for chronic lymphocytic leukemia (CLL) patients treated with chemo-immunotherapy, but recurrent disease is common and chemotherapy may select for drug-resistant leukemic subclones and amplify immune suppression. The clinical efficacy of immunotherapy in CLL has been demonstrated after allogeneic stem cell transplantation (with all its potential toxicity) where long-term disease control has been achieved by exploiting a graft-versus-leukemia effect.[2] Expanding immunotherapeutic treatment options should aid identification of potentially curative therapies for CLL and other malignancies. Cellular and humoral immune defects impair immunotherapeutic approaches. CLL cells are dysfunctional antigen-presenting cells (APCs) and can actively suppress T-cell cytoskeletal signaling pathways and function.[3] Repair and reversal of autologous immune dysfunction in CLL should reprogram patients' immune system to elicit antitumor responses.

The most promising immunotherapy in CLL has been using adenovirus-delivered CD154 gene therapy.[4,5] Transduction of CD154 into patient CLL cells ex vivo, followed by systemic reintroduction, induces CD40 activation of both transduced and nontransduced CLL cells, which stimulate autologous leukemia-specific T cells, reduce leukemia cell counts, and induce development of antibodies to the CLL tumor-specific antigen ROR1. CD154-ligand treatment also induces expression of death receptors CD95 and DR5, and p73 that can render CLL cells sensitive to TRAIL-mediated apoptosis and fludarabine-based therapies.[6] The impaired immune response in CLL patients likely explains why CD154 gene therapy has failed to achieve more durable clinical responses.

The article by Lapalombella et al identifies lenalidomide (Revlimid; Celgene) as a pharmacologic agent that can mimic a CD154 gene therapy activation phenotype in CLL and reverse humoral immune tolerance. Lenalidomide is clinically effective as a single agent in relapsed or refractory CLL,[7] but no direct in vitro proapoptotic effect has been observed using primary CLL cells. The exact mechanisms of action of lenalidomide in hematologic malignancies are not fully defined but include activation of cellular and innate immunity, and blocking angiogenic and stromal cell activity.

Lapalombella and colleagues demonstrate that lenalidomide exposure induces CD154 transmembrane protein expression on primary CLL cells. Mechanistic data show that the drug enhances CD154 mRNA gene transcription via NFATc1/NF-κB complex binding to the CD154 promoter and also through downstream mRNA stabilization, both of which are dependent on the PI3-kinase pathway. This study also presents elegant functional coculture data showing that lenalidomide-induced surface expression of CD154 on CLL cells promotes immunoglobulin production by normal B cells and increases BID, DR5, and p73 expression as seen with CD154 gene therapy. In vivo data show that patients receiving lenalidomide exhibit the CD154 gene therapy CLL phenotype, and in

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