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. ■

REFERENCES

1. Mansson R, Zandi S, Welinder E, et al. Single-cell analysis of the common lymphoid progenitor compartment reveals functional and molecular heterogeneity. *Blood*. 2010; 115(13):2601-2609.
2. Nutt SL, Heavey B, Rolink AG, Busslinger M. Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature*. 1999;401(6753):556-562.
3. Rumfelt LL, Zhou Y, Rowley BM, Shinton SA, Hardy RR. Lineage specification and plasticity in CD19- early B cell precursors. *J Exp Med*. 2006;203(3):675-687.
4. Mansson R, Zandi S, Anderson K, et al. B-lineage commitment prior to surface expression of B220 and CD19 on hematopoietic progenitor cells. *Blood*. 2008;112(4): 1048-1055.
5. Welner RS, Esplin BL, Garrett KP, et al. Asynchronous RAG-1 expression during B lymphopoiesis. *J Immunol*. 2009;183(12):7768-7777.
6. Inlay MA, Bhattacharya D, Sahoo D, et al. Ly6d marks the earliest stage of B-cell specification and identifies the branchpoint between B-cell and T-cell development. *Genes Dev*. 2009;23(20):2376-2381.
7. Pongubala JM, Northrup DL, Lancki DW, et al. Transcription factor EBF restricts alternative lineage options and promotes B cell fate commitment independently of Pax5. *Nat Immunol*. 2008;9(2):203-215.

● ● ● LYMPHOID NEOPLASIA

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

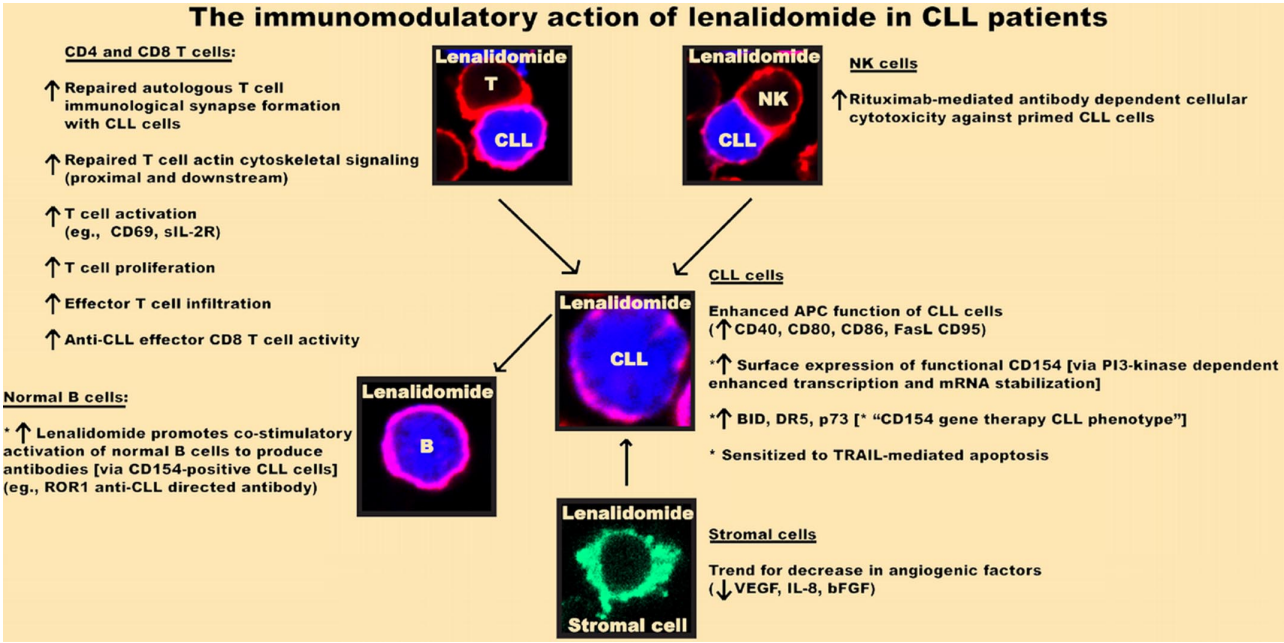
In this issue of *Blood*, Lapalombella and colleagues present evidence that the immunomodulatory drug lenalidomide (Revlimid) may repair and reverse the humeral defect in CLL.[1]

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



Lenalidomide (Revlimid) repairs and reverses cellular and humoral immune dysfunction in CLL. This agent enhances autologous T-cell immunologic synapse formation and functional activity (repairs impaired T-cell actin cytoskeletal signaling). NK-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CLL cells is enhanced by preincubation of NK cells with lenalidomide before exposure to rituximab-exposed CLL cells. Lenalidomide enhances APC function of CLL cells (up-regulation of costimulatory molecules). Exposure of drug increases functional surface CD154 expression on CLL cells, inducing a CD154 gene therapy activation phenotype. Importantly, lenalidomide-induced CD154-positive CLL cells promote antibody (IgM and IgG) production by normal B cells. The immunomodulatory effect of lenalidomide on stromal cells, nurse-like cells, and angiogenic status remains to be fully elucidated.

one patient, development of polyclonal hypergammaglobulinemia with increasing titers of the CLL-specific antibody ROR1. It would have been very informative to strengthen the in vivo patient data showing that lenalidomide can reverse humoral tumor tolerance. The authors suggest that the reason only a single case report identified recovery of immunoglobulins and development of ROR1 tumor-specific antibodies was that all the other patients received prior rituximab, which depletes normal B cells as well as malignant cells.

CLL-induced T-cell dysfunction can be reversible with lenalidomide.[3] This agent can repair autologous T-cell recognition and formation of the immunologic synapse with CLL cells leading to enhanced cytolytic killing. Pretreatment of autologous natural killer (NK) cells with lenalidomide enhances rituximab-mediated antibody-dependent cellular cytotoxicity of CLL[8] at clinically relevant lenalidomide concentrations (0.5μM) that repair and reverse the humoral and cellular immune defects. This relatively low pharmacologic dose might help minimize toxicity associated with the drug-induced tumor flare reaction. The ability of lenalidomide to reverse and repair both T-cell and humoral immune dysfunction provides strong evidence that a major mode of action of this agent in CLL is immunomodulatory (see figure). The combined results of rigorous correlative science functional studies suggest that the timing of lenalidomide should be carefully designed to ensure that immunosuppressive chemotherapeutic therapies and rituximab do not block the immunomodulatory action of this exciting new agent in CLL. Further work in CLL and cross-fertilization of results using lenalidomide in other B-cell malignancies should maximize the clinical application of this agent. Lenalidomide's immunotherapeutic activity suggests that it should be considered in combination with vaccination in clinical trials of immunotherapy, especially in light of the current findings that this drug may reverse the humoral immune defect in CLL.

Conflict-of-interest disclosure: J.G.G. has received honoraria from Celgene for work on advisory boards. A.G.R. declares no competing financial interests. ■

REFERENCES

1. Lapalombella R, Andritsos L, Liu Q, et al. Lenalidomide treatment promotes CD154 expression on CLL cells and enhances production of antibodies by normal B cells through a PI3-kinase–dependent pathway. *Blood.* 2010; 115(13):2619-2629.

2. Gribben JG. Stem cell transplantation in chronic lymphocytic leukemia. *Biol Blood Marrow Transplant.* 2009; 15(1 suppl):53-58.

3. Ramsay AG, Johnson AJ, Lee AM, et al. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. *J Clin Invest.* 2008;118(7):2427-2437.

4. Wierda WG, Cantwell MJ, Woods SJ, Rassenti LZ, Prussak CE, Kipps TJ. CD40-ligand (CD154) gene therapy for chronic lymphocytic leukemia. *Blood.* 2000;96(9): 2917-2924.

5. Fukuda T, Chen L, Endo T, et al. Antisera induced by infusions of autologous Ad-CD154-leukemia B cells identify ROR1 as an oncofetal antigen and receptor for Wnt5a. *Proc Natl Acad Sci U S A.* 2008;105(8):3047-3052.

6. Dicker F, Kater AP, Fukuda T, Kipps TJ. Fas-ligand (CD178) and TRAIL synergistically induce apoptosis of CD40-activated chronic lymphocytic leukemia B cells. *Blood.* 2005;105(8):3193-3198.

7. Ferrajoli A, Lee BN, Schlette EJ, et al. Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. *Blood.* 2008;111(11):5291-5297.

8. Lapalombella R, Yu B, Triantafillou G, et al. Lenalidomide down-regulates the CD20 antigen and antagonizes direct and antibody-dependent cellular cytotoxicity of rituximab on primary chronic lymphocytic leukemia cells. *Blood.* 2008;112(13):5180-5189.