Commentary

Chronic Lymphocytic Leukemia: "Cinderella" Is Becoming a Star

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It has taken time for the status of chronic lymphocytic leukemia (CLL) to change within the scientific community. CLL, characterized by the accumulation of seemingly innocent long-lived monoclonal B cells exhibiting mature morphologies, has long been considered the "Cinderella" of blood cancers. CLL is receiving increasing attention from biologists and clinicians, however, because understanding of this disease may elucidate the association between lymphoid tumous and autoimmunity as well as help to define the relationships between antigen stimulation and malignant transformation.

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) has been considered the "Cinderella" of blood cancers, receiving less attention than related diseases. Now, however, CLL is a focus of investigation by biologists and clinicians because characteristics of this disease may shed light on the association between lymphoid tumours and autoimmunity as well as help to define the relationships between antigen (Ag) stimulation and malignant transformation.

CHARACTERISTICS OF CLL CELLS

CLL B cells are endowed with a functional B-cell receptor (BCR) that allows Ag interaction (1,2). The cell of origin of CLL has been largely ignored even if its phenotype closely resembles lymphocytes detectable in the marginal zone of secondary lymphoid organs (3). Somatic mutations of the *Immunoglobulin Heavy Chain Variable* (*IGHV*) genes are present in at least half of CLL patients (4,5), indicating that in some cases antigenic pressure may influence the development of CLL. The presence or absence of *IGHV* somatic mutations is clinically relevant because prognosis is significantly better in cases in which mutations are present than in cases in which they are absent (4,5). CLL patients exhibit a biased use of *IGHV* genes, and subsets of patients can be identified who carry closely homologous if not identical ("stereotyped") complementarity-determining region 3 (CDR3) sequences on heavy and light chains (6–10). CDR3 regions are unique for each B lymphocyte and its progeny. The probability of two individual B cells expressing identical BCRs is extremely low (10^{-9} to 10^{-12}). Therefore the remarkable BCR similarity detected in more than 25% of unrelated and geographically distant CLL cases (9,10) cannot be accounted for by pure chance. Stereotyped CDR3 sequences are more frequently observed in CLL cases without mutations than in cases with mutations [approximately 40% versus 10%, (10)], indicating that antigenic exposure may be relevant in the pathogenesis of CLL, irrespective of *IGVH* mutational status. Results of expression profiling and cytofluorography as well as functional data document that all CLL cases, regardless of their *IGVH* mutational status, show the signatures of BCR-mediated stimulation, express membrane markers of cellular activation, and secrete a wide variety of cytokines similar to those of Ag-activated B cells (1–3,11). Numerous studies have investigated these data, and the results of these studies indicate that CLL B cells are Ag experienced, suggesting a central role for the recognition of a limited set of structurally similar epitopes in the selection and growth of leukemic clones.

Chronic lymphocytic leukemia (CLL), long considered the "Cinderella" of blood cancers, is receiving increasing attention from biologists and clinicians.

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AG STIMULATION IN B-CELL MALIGNANCIES

Increasing evidence indicates that chronic Ag stimulation favors the development and progression of several chronic B-cell malignancies, especially of marginal-zone origin (12), the classical example being the onset of gastric MALT lymphomas in the context of chronic *Helicobacter pylori* infection (13). Self-Ag may be involved as well, as in the case of Sjogren syndrome or of Hashimoto thyroiditis, in which persistent immune system stimulation, caused by autoAg, underlies lymphoma development in salivary glands or within thyroid tissue, respectively.

Now we arrive at the heart of the CLL conundrum: which Ag are involved, and where and how? A corollary of this question is whether target cells have experienced Ag stimulation before the occurrence of transforming events that lead to malignancy or whether they are continuously exposed to Ag stimulation. If so, then Ag intervention may influence not only the onset of CLL but also its progression. The relevance of these issues is underscored by their potential therapeutic implications.

Monoclonal recombinant antibodies (Abs) derived from several subsets of BCR-stereotyped CLL cells have been shown to bind to intracellular autoAg (14). Recently two independent studies (15,16) have analyzed a large panel of Epstein-Barr virus–transformed CLL cell lines and primary CLL cells that use different *VHIG* genes and cover mutated and unmutated cases, some presenting CDR3-stereotyped receptors. Both studies showed that most if not all cases of CLL involve production of polyreactive monoclonal Abs that react with a number of novel autoAg targets, including cytoskeletal proteins, phosphorylcholine-containing structures, and oxidized low-density lipoproteins. Interestingly, most Ag in their native forms are localized in the cytoplasm, whereas in the prototype autoimmune disorder systemic lupus erythematosus, autoAg are nuclear. Two categories of Ag have been detected: (*a*) native molecular motifs, which are located in the cytoplasm of viable cells but relocate when the cell undergoes apoptosis and become exposed on apoptotic cells/blebs, and (*b*) neo-Ags, which are generated by oxidation during the apoptotic process. These properties explain why CLL monoclonal Abs react with apoptotic lymphocytes but not with the same cells when viable. Remarkably, some of these Ag are also exposed on the surface of common bacteria.

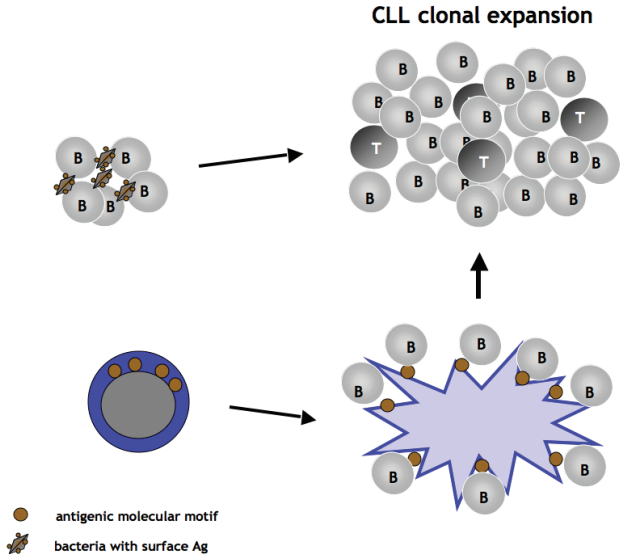

Figure 1. Antigenic molecular motif targets of polyreactive monoclonal antibodies produced by CLL cells are cytoplasmic in viable lymphocytes (bottom left), but relocate to the surface when the cells undergo apoptosis (bottom right) and are also exposed on the surface of bacteria. CLL cell growth might be fuelled by the interaction of CLL cell surface polyreactive antibodies with these autoantigens.

DISCUSSION

The relevance of these findings is three-fold. First, they provide the proof of principle that natural Abs in humans present similarly to those in mice, in which natural autoAbs have been described in different settings (17–19), such as autoimmunity and removal of senescent cells and bacteria. This similarity helps elucidate the nature of the normal cell precursor of CLL in humans. These results suggest that CLL cells derive from a subset of cells normally involved in eliminating cellular debris, scavenging apoptotic cells, and providing the first line of defense against pathogenic bacteria. Thus, interest in human CD5+ B cells, their relationships with mouse B1 cells, and their position within the complex cellular milieu of the marginal zone in secondary lymphoid organs is rejuvenated.

The second important feature of these findings is that the data reported by Myrinder *et al*. (15) and Catera *et al*. (16) improve our understanding of the mechanism that favors clonal CLL expansion by raising the intriguing issue that the progression of CLL may be fuelled by ongoing apoptotic processes, possibly in synergy with bacterial infections (Figure 1). These data are well in keeping with the observation that bacterial infections of the respiratory tract increase the risk of CLL (20) and may even suggest that CLL cells themselves—once they undergo apoptosis within infiltrated peripheral tissues—might be involved in self-perpetuating the disease by providing autoantigenic targets. It will be important to understand whether these novel autoAgs favor expansion of an existing genetically abnormal cell popula-

tion whose underlying genetic defect is brought to light by Ag stimulation.

The third important point is that the nature of the Ag, together with BCR affinity, may shape the clinical course of CLL. CLL cells differ significantly in their *in vitro* capacity to signal through the BCR: some CLL cases (most unmutated) carry more competent BCRs, whereas others (usually mutated) appear to be unresponsive (21). In the former cases persistent Ag stimulation might promote malignant cell survival and growth, and in the latter Ag interaction might lead to receptor desensitization and an anergic state (22). The role of Ag stimulation in the clinical course of CLL is therefore a concept worthy of further investigation by clinicians as well as scientists.

DISCLOSURE

We declare that the authors have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

REFERENCES

1. Caligaris-Cappio F, Ghia P. (2008) Novel insights in chronic lymphocytic leukemia: are we coming closer to understanding the pathogenesis of the disease? *J. Clin. Oncol.* 26:4497–503.
2. Stevenson FK, Caligaris-Cappio F. (2004) Chronic lymphocytic leukemia: revelations from the B-cell receptor. *Blood* 103:4389–95.
3. Chiorazzi N, Rai KR, Ferrarini M. (2005) Chronic lymphocytic leukemia. *N. Engl. J. Med.* 352:804–15.
4. Hamblin TJ, *et al.* (1999) Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood* 94:1848–54.
5. Damle RN, *et al.* (1999) Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood* 94:1840–7.
6. Ghiotto F, *et al.* (2004) Remarkably similar antigen receptors among a subset of patients with chronic lymphocytic leukemia. *J. Clin. Invest.* 113:1008–16.
7. Messmer BT, *et al.* (2004) Multiple distinct sets of stereotyped antigen receptors indicate a role for antigen in promoting chronic lymphocytic leukemia. *J. Exp. Med.* 200:519–25.
8. Ghia P, *et al.* (2006) Geographic patterns and pathogenetic implications of IGHV gene usage in chronic lymphocytic leukemia: the lesson of the IGHV3–21 gene. *Blood* 105:1678–85.
9. Stamatopoulos K, *et al.* (2006) Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: pathogenetic implications and clinical correlations. *Blood* 109:259–70.
10. Murray F, *et al.* (2008) Stereotyped patterns of somatic hypermutation in subsets of patients with chronic lpymphocytic leukemia: implications for the role of antigen selection in leukemogenesis. *Blood* 111:1524–33.
11. Klein U, *et al.* (2001) Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. *J. Exp. Med.* 194:1625–38.
12. Suarez F, *et al.* (2006) Infect.ion-associated lymphomas derived from marginal zone B cells: a model of antigen-driven lymphoproliferation. *Blood* 107:3034–44.
13. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson P. (1991) Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 338, 1175–6.
14. Herve M, *et al.* (2005) Unmutated and mutated chronic lymphocytic leukemias derive from self-reactive B cell precursors despite expressing different antibody reactivity. *J. Clin. Invest.* 115:1636–43.
15. Myrinder AL, *et al.* (2008) A new perspective: molecular motifs on oxidized LDL, apoptotic cells, and bacteria are targets for chronic lymphocytic leukemia antibodies. *Blood* 111:3838–48.
16. Catera R, *et al.* (2008) Chronic lymphocytic leukemia cells recognize conserved epitopes associated with apoptosis and oxidation. *Mol. Med.* 14:665–74.
17. Martin T, Duffy SF, Carson DA, Kipps TJ. (1992) Evidence for somatic selection of natural autoantibodies. *J. Exp. Med.* 175:983–91.
18. Carroll MC. (2004) A protective role for innate immunity in systemic lupus erythematosus. *Nat. Rev. Immunol.* 4:825–31.
19. Binder CJ, *et al.* (2003) Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between Streptococcus pneumoniae and oxidized LDL. *Nat. Med.* 9:736–43.
20. Landgren O, *et al.* (2007) Respiratory tract infections and subsequent risk of chronic lymphocytic leukemia. *Blood* 109:2198–201.
21. Lanham S, *et al.* (2003) Differential signaling via surface IgM is associated with VH gene mutational status and CD38 expression in chronic lymphocytic leukemia. *Blood* 101:1087–93.
22. Muzio M, *et al.* (2008) Constitutive activation of distinct BCR-signaling pathways in a subset of CLL patients: a molecular signature of anergy. *Blood* 112:188–95.