When chronic lymphocytic leukemia (CLL) was last reviewed in the Journal, it was considered a homogeneous disease of immature, immune-incompetent, minimally self-renewing B cells, which accumulate relentlessly because of a faulty apoptotic mechanism. In the past decade, these views have been transformed by a wealth of new information about the leukemic cells. CLL is now viewed as two related entities, both originating from antigen-stimulated mature B lymphocytes, which either avoid death through the intercession of external signals or die by apoptosis, only to be replenished by proliferating precursor cells (Table 1).

**Table 1**

<table>
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<tr>
<th><strong>NORMAL B LYMPHOCYTES</strong></th>
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B lymphocytes mature in the bone marrow (Fig. 1) and in the process rearrange immunoglobulin variable (V) gene segments to create the code for an immunoglobulin molecule that serves as the B-cell receptor for antigen. When an antigen of adequate affinity engages the receptor, the cell enters a germinal center in lymphoid follicles, where, as a centroblast, it rapidly divides and its V genes undergo somatic hypermutation (Fig. 2). This process introduces mutations in the rearranged V<sub>H</sub> and V<sub>L</sub> gene segments that code for the binding site of the receptor. Through these mutations, the receptors of the descendant B cells, called centrocytes, acquire new properties. Cells with receptors that have enhanced antigen-binding affinity proliferate in the presence of the antigen, whereas centrocytes with receptors that no longer bind the antigen or do bind autoantigens are normally eliminated.

This stimulation and selection pathway usually requires the help of T lymphocytes and occurs in germinal centers, the structure of which ensures the selection of antigen-avid B cells. However, the process can proceed without T cells and outside germinal centers, in the marginal zones around lymphoid follicles, most often in response to carbohydrates of encapsulated bacteria or viruses (Fig. 2). Both processes lead to the development of plasma cells or memory (antigen-experienced) B cells.

Concomitant with B-cell activation, the proteins on the surface of the B cell change. These modifications help activated B cells to interact with other cells and soluble mediators and thereby increase in number or mature into antibody-producing plasma cells. One surface molecule that supports B-cell interactions and differentiation is CD38. CD38 has adenosine diphosphate–ribose cyclase activity, and under certain circumstances augments signaling of B-cell receptors and delivers signals that regulate the apoptosis of B cells.

Signals received through B-cell surface receptors are transferred to the nucleus by a cascade of interacting molecules whose structures are temporarily modified during the process. These modifications frequently involve the attachment of phosphate groups to tyrosines of target proteins by specific enzymes. The enzymes involved in the initial phases of the signaling cascade include Syk and Lyn, members of the Src family.
of protein tyrosine kinases. For T lymphocytes, the zeta-chain–associated protein 70 (ZAP-70) is a crucial player with similar activity.

**CLL AND THE BIOLOGY OF LEUKEMIC LYMPHOCYTES**

The monoclonal population of B cells in CLL expresses CD19, CD5, and CD23 and has reduced levels of membrane IgM, IgD, and CD79b, a phenotype of mature, activated B lymphocytes. The pathological features of the lymph node are those of a small lymphocytic lymphoma.

Some patients with CLL survive for many years without therapy and eventually succumb to unrelated diseases, whereas others have a rapidly fatal disease despite aggressive therapy. Recognizing this heterogeneity, Rai and colleagues and Bi- net and colleagues devised staging systems for use in assessing the extent of disease in an individual patient. These systems remain the cornerstones on which decisions regarding medical follow-up and treatment are built, but they fail to predict the course of the disease in patients in whom CLL is diagnosed in early stages.

Within the past decade, CLL has been shown to be a remarkably diverse disorder. Its heterogeneity reflects differences in the mutation status of V genes, and profiling of the expression of genes genomewide. When cases of CLL were divided into categories on the basis of these differences, profoundly disparate clinical courses were revealed. Patients with clones having few or no V-gene mutations or with many CD38+ or ZAP-70+ B cells had an aggressive, usually fatal course, whereas patients with mutated clones or few CD38+ or ZAP-70+ B cells had an indolent course.

Mutations of V genes are detected by comparing DNA sequences of the genes in B cells with corresponding genes in the germ line. A sequence that differs from its germ-line counterpart by 2 percent or more is defined as mutated. According to this criterion, CLL cases are divisible into two groups: in the first, leukemic cells have rearranged V genes with 2 percent or more mutations (“mutated” CLL); in the second, there are few or no mutations (less than 2 percent; “unmutated” CLL). The presence of V-gene mutations and the presence of few CD38+ cells do not always correlate.

ZAP-70 is an intracellular protein that promulgates activation signals delivered to T lymphocytes and natural killer cells by their surface receptors for antigen (Fig. 3). It is rarely present in normal B cells but has been found in B cells from patients with CLL. When the expression of ZAP-70 is manipulated experimentally in B cells, it can facilitate signal transmission down the pathway initiated by antigen engagement with the B-cell receptor. Gene-expression profiles indicate that unmutated CLL cells express more ZAP-70 mRNA than do mutated CLL cells. The analysis of DNA sequences to determine the status of immunoglobulin V-gene mutations is laborious and not performed routinely in clinical laboratories, whereas

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**Table 1. A Comparison of Historical and Current Views of CLL.**

<table>
<thead>
<tr>
<th>Historical View</th>
<th>Current View</th>
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<tbody>
<tr>
<td>CLL is a clinically heterogeneous disease with a homogeneous cellular origin.</td>
<td>CLL is a clinically heterogeneous disease originating from B lymphocytes that may differ in activation, maturation state, or cellular subgroup.</td>
</tr>
<tr>
<td>CLL is a disease derived from naive B lymphocytes.</td>
<td>CLL is a disease derived from antigen-experienced B lymphocytes that differ in the level of immunoglobulin V-gene mutations.</td>
</tr>
<tr>
<td>Leukemic-cell accumulation occurs because of an inherent apoptotic defect involving the entire mass of leukemic cells.</td>
<td>An inherent apoptotic defect involving the entire mass of leukemic cells is unlikely to exist initially. Leukemic-cell accumulation occurs because of survival signals delivered to a subgroup of leukemic cells from the external environment through a variety of receptors (e.g., B-cell receptors and chemokine and cytokine receptors) and their cell-bound and soluble ligands.</td>
</tr>
<tr>
<td>CLL is a disease of accumulation.</td>
<td>CLL is a disease of accumulation with a higher associated level of proliferation than was previously recognized.</td>
</tr>
<tr>
<td>Prognostic markers identify patients at various risk levels (low, intermediate, or high in the Rai staging categories and A, B, or C in the Binet categories) with an acknowledged heterogeneity in clinical outcomes among patients in the low- and intermediate-risk categories.</td>
<td>New molecular and protein markers identify patients within the low- and intermediate-risk categories who follow different clinical courses.</td>
</tr>
<tr>
<td>Therapy is based largely on clinical observations and trial-and-error methods.</td>
<td>New findings provide clues to discrete targets for developing hypothesis-driven and effective therapeutic agents.</td>
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</table>

*“Current view” refers to our understanding of the biology and derivation of CLL cells as it has evolved during the past 10 years.*
testing for ZAP-70, when appropriately standardized, can more readily serve as a clinical test.\textsuperscript{31,33-35}

\section*{THE BIOLOGY OF LEUKEMIC LYMPHOCYTES AND THE CLINICAL COURSE OF CLL}

\subsection*{Inducing Factors in CLL}

Chromosomal translocations involving oncogenes frequently cause B-cell lymphomas.\textsuperscript{46} CLL is a special case, however, because chromosomal translocations are rare, and no unifying mutations have been identified. Yet the monoclonal nature of the B lymphocytes that proliferate in this disease imply that inducing lesions must exist in the progenitor clone.

Cytogenetic lesions are rare in the leukemic clone early in the course of the disease and therefore are not likely to be inducing factors. Nevertheless, some appear as the disease progresses. The most common is a deletion at 13q14.3, which occurs in more than 50 percent of cases over time.\textsuperscript{47} This deleted region contains a nontranscribed gene\textsuperscript{48} and two micro-RNA genes.\textsuperscript{49} Micro-RNA is made normally by cells, including B lymphocytes,
and regulate the functions of many genes, some of which may have relevance to cancer in general and CLL in particular. Two micro-RNA genes located at 13q14 are deleted or down-regulated in most cases of CLL. The frequency of this deletion implies that it confers a selective advantage, possibly predisposing B-cell clones to undergo additional mutations.

The most ominous alterations are deletions at 11q22–23, 17p13, and 6q21. Although the genes that are involved in these lesions are unknown, it is likely that p53 is included in the deletion at 17p13 and that the ataxia–telangiectasia mutated (ATM) gene is involved in the deletion at 11q22–23. Both genes regulate apoptosis and confer resistance to chemotherapy. These deletions are relatively frequent in unmutated CLL cases with a poor outcome.

Interestingly, TCL-1, located at 14q32.1 and involved in the pathogenesis of T-cell prolymphocytic leukemia, is expressed in CLL. In mice that were genetically manipulated to overexpress TCL-1 in B cells, a leukemia or lymphoma of CD5+ B cells developed that was reminiscent of CLL.
attractive candidates for an inducing factor, abnormalities of TCL-1 or its regulation have not been identified in patients with CLL.

**PROMOTING FACTORS IN CLL**

New evidence suggests that antigenic stimulation, along with interactions with accessory cells and cytokines, is a promoting factor that stimulates proliferation of CLL cells and allows them to avoid apoptosis. These effects may differ in distinct CLL subgroups and thereby lead to the disparity in clinical outcomes among individual cases.

**Inferring the Role of Antigenic Stimulation from B-Cell Receptors**

The B-cell receptors of CLL cells from various patients are often structurally very similar, suggesting that the antigens these receptors bind are similar and relevant to the pathogenesis of CLL. The extent of similarity varies among groups of patients. In some cases, there are shared features in the portion of the antigen-binding site contributed by the H chain (VH, D, and JH genes). In these cases, each VH gene exhibits special patterns of mutations and preferential combinations with particular D or JH segments, which generate distinct features in the antigen-binding pocket. These VH-DJH rearrangements and characteristics of antigen-binding pockets differ from the much broader diversity found in B cells from normal persons.

In other groups of cases, the structural similarity of the receptors involves the entire antigen-binding site, coded by both the H and L chains (VH, D, JH, and VL and JL genes). In these instances, the receptors from various patients are very similar or virtually identical. As many as 10 percent of all CLL cases fall into distinct categories branded by receptors with structurally similar antigen-binding pockets; most are of the unmutated, poor-outcome type. These findings are very striking since, given the number of possible combinations of V-gene segments that can encode antigen-binding domains, one would not expect to find 2 cases of CLL with such structurally similar B-cell receptors in more than 1 million cases.

These cases suggest that a limited set of antigens promotes division of the leukemic cells, increasing the likelihood of dangerous DNA mutations. What are these promoting antigens? They are unknown, but it is possible that latent viruses or commensal bacteria repetitively activate particular B-cell clones through the B-cell receptor. CLL would result, directly or indirectly, from specific infections and would be perpetuated by them — in a manner similar to the gastric lymphomas that evolve in response to *Helicobacter pylori*.

Alternatively, environmental antigens or autoantigens could provoke clonal expansion. CLL cells frequently have polyreactive receptors, which bind multiple antigens, including autoantigens, allowing stimulation by both autoantigens and microbial antigens. This mechanism is plausible for...
unmutated CLL and also for a few cases of mutated CLL, since many unmutated\textsuperscript{71,72} and some mutated\textsuperscript{73} immunoglobulin V genes encode such polyreactive receptors; this immune-stimulation mechanism is in keeping with the view that the basis of CLL is autoimmunization.\textsuperscript{74}

As constitutive low-level signaling is delivered through the B-cell receptor in normal B lymphocytes, perhaps to maintain the memory response and the B-cell repertoire,\textsuperscript{75,76} antigen may not be necessary to continue clonal expansion — antigen-independent triggering might occur through the B-cell-receptor signaling pathway because of another genetic lesion.

**Signal Transduction after Antigen Engagement**

For antigenic stimulation to underlie clonal expansion, the B-cell receptor must propagate an efficient signal to the cell nucleus (Fig. 3). Leukemic cells from different CLL subgroups can differ in this capacity. Cross-linking B-cell receptor molecules with antibodies to IgM in vitro mimics the engagement of antigens with B-cell receptors and transmits signals to the cell nucleus in approximately 50 percent of cases of CLL.\textsuperscript{77-81} This phenomenon seems to occur mainly in unmutated CLL,\textsuperscript{77,78,82} but more patients need to be studied for this to be confirmed. CLL cells that do not respond to stimulation from the B-cell receptor may be frozen at a stage at which even normal B lymphocytes would be unresponsive to antigen.\textsuperscript{41} Alternatively, these cells could be anergic, possibly because of previous antigenic experience.\textsuperscript{42} Finally, these CLL cells may have become incapable of responding to antigens because of changes in the structure of their B-cell receptors caused by somatic mutations or an inability of the cells to come into contact with relevant antigens in vivo.\textsuperscript{41} Considering the number of cells that make up the leukemic clone in many patients (10\textsuperscript{11} to 10\textsuperscript{12}), it is likely that only a fraction of the members of the clone could encounter the antigen, especially if they are restricted to discrete anatomical locations or compartments.

Other, not mutually exclusive, possibilities to explain the lack of B-cell–receptor signaling include reduced numbers of B-cell–receptor molecules,\textsuperscript{17} uncoupling of the B-cell receptor from accessory molecules necessary for effective signal transduction,\textsuperscript{83-85} and mutations in these accessory structures.\textsuperscript{86} It is interesting to note that responsiveness to stimuli delivered through surface IgD is frequently maintained in these cases.\textsuperscript{82,87}

**Consequences of Signal Transduction through the B-Cell Receptor**

Once signal transduction is initiated by the B-cell receptor, B lymphocytes progress into the cell cycle or die. Cross-linking of surface IgM in CLL cells that can transduce a signal can cause\textsuperscript{88} or prevent\textsuperscript{88} apoptosis (Fig. 3), whereas cross-linking surface IgD invariably prevents apoptosis.\textsuperscript{12,81} This difference is unexpected, because the two surface isotypes express the same clone-specific antigen-binding site and provide concordant signals in mature B cells. The final outcome of B-cell receptor signaling in an individual CLL cell, therefore, depends on the balance between signals mediated by the two molecules.

**Signals from the Microenvironment**

Signals that are delivered by direct cell contact or soluble factors, which may or may not occur concomitantly with B-cell–receptor engagement, probably propagate the growth of CLL cells (Fig. 3). Interactions with stromal cells\textsuperscript{89} or nurse-like cells\textsuperscript{90} or interactions between CD38 and its natural ligand CD31\textsuperscript{91} rescue CLL cells from apoptosis in vitro and probably do the same in vivo. Activated T cells or other cells expressing CD40 ligand also support the growth of CLL cells.\textsuperscript{92} Finally, cytokines such as interleukin-4 and vascular endothelial growth factor\textsuperscript{93-95} and chemokines such as SDF-1\textsuperscript{96} (particularly in the presence of stromal cells) support the expansion of CLL clones.

These signals tip the balance between antiapoptotic signals and proapoptotic signals in favor of cell survival. There is up-regulation of the antiapoptotic genes BCL2, survivin, and MCL1 in leukemic cells.\textsuperscript{92,97} Rescue from apoptosis and facilitation of cell growth may occur preferentially in lymph-node pseudofollicles and bone marrow clusters,\textsuperscript{89,98} evidenced by expression of the cycling cell marker Ki-67 by the leukemic cells in these sites.\textsuperscript{92} Because growth of the clone depends on a variety of interactions with the environment, variations in the requirements for these interactions on part of the leukemic cells may be responsible for changes in the clinical course.\textsuperscript{99,100}

**Appearance and Evolution of New Genetic Mutations**

The emergence of new, aggressive clonal variants, which can worsen the disease, requires proliferation of the leukemic clone. In vivo studies using radioactive and nonradioactive means suggest that CLL cells are more dynamic than is usually appreci-
ated. C.1-103 CLL cells have surprisingly brisk birth rates, ranging from about 0.1 to more than 1.0 percent of the clone per day. C.1-103 If the total clonal burden of a typical patient with CLL is approximately $10^{12}$ cells, these birth rates point to the daily production of some $10^9$ to $10^{10}$ new leukemic cells.

These rates of cell division are sufficient to permit clonal variants to emerge. Indeed, there is an association between brisk birth rates of CLL cells and progressive disease. C.103 The rate of birth of leukemic cells, therefore, may be more relevant clinically than is either the blood lymphocyte count or the physical examination, since the lymphocyte count reflects the proliferative capacity of the leukemic cells and their potential to promote new DNA lesions, whereas the sizes of the lymph nodes and spleen on physical examination reflect a balance between cell proliferation and cell death. These findings may explain why telomeres, which cap and protect the ends of chromosomes but shorten with each cell division, are smaller in cells from patients in CLL subgroups that have poor outcomes. C.104,105

**A UNIFYING HYPOTHESIS FOR THE DEVELOPMENT, GROWTH, AND EVOLUTION OF CLL**

**GROWTH AND EVOLUTION OF CLL CELLS**

The above considerations suggest a plausible model on which to build future hypotheses and studies. Stimulatory and growth signals from the environment of CLL cells allow them to avoid apoptosis and proliferate. These signals are delivered by the B-cell receptor, receptors for cytokines or chemokines and other ligands, and direct contact with accessory and stromal cells. The major growth effects mediated by the B-cell receptor appear to occur in cases in which the receptor permits binding of autoantigens and maintains the capacity to transmit stimulatory signals to the cell nucleus (i.e., those with unmutated and, to a lesser degree, mutated CLL B-cell receptors).

This model excludes an intrinsic apoptotic defect in all members of the leukemic clone. Indeed, in vitro observations demonstrate the absence of lesions in the major apoptotic pathways. C.87,106 Whether continued cell division is facilitated by external signals or not, the level of B-cell turnover in vivo can suffice to promote the development and outgrowth of subclones with new genetic lesions and a growth advantage (Fig. 4).

**DEVELOPMENT OF CLL FROM NORMAL B LYMPHOCYTES**

Many normal B lymphocytes with unmutated V genes produce antibodies capable of binding multiple antigens (e.g., carbohydrates, nucleic acids, and phospholipids) C.107 and of providing the first line of defense against microorganisms. If one of these cells contained or developed a genetic abnormality that allows it to resist restraint on clonal size (e.g., an initial inducing lesion), then this cell would be primed for leukemic transformation (Fig. 4A). Foreign antigens and autoantigens then could be important stimuli for the development of CLL. C.109 B cells with such unmutated polyreactive B-cell receptors could expand and convert to CLL cells with repetitive exposure to microbes and to autoantigens (Fig. 4B). C.110-113 A similar mechanism may underlie the origin of mutated CLL, because V-gene mutations can occur without T-cell help C.5,7,8 outside of germinal centers, and these mutations can sometimes favor autoreactivity. C.73 Such expansion would stop if V-gene mutations altered the structure of the B-cell receptor in a way that caused loss of binding to the stimulatory antigen (i.e., the development of “clonal ignorance”) (Fig. 4C).

This hypothesis implies that such expansions should be detectable in healthy patients. Recent studies suggest that small numbers of clonal B cells with the characteristics of CLL cells exist in the blood of approximately 3.5 percent of disease-free persons C.114,115 and in an even higher proportion of first-degree relatives of patients with CLL. C.116 Although studies of the B-cell receptors of these B-lymphocyte expansions are limited, initial information suggests that they are monoclonal and use some of the genes that encode the B-cell receptors of CLL clones.

**FROM WHICH SUBPOPULATION DO CLL CELLS DEVELOP?**

Since CLL cells resemble activated B lymphocytes, C.117 their cellular origin cannot be deduced solely from phenotypic analyses, a fact that makes it difficult to draw a direct parallel with B1 cells described in mice. C.118 However, certain functional features may help delineate their origin. Normal adult B cells that produce autoantibodies and antibodies against bacterial or viral carbohydrates reside in the marginal zone. It is possible that marginal-zone B cells are the precursors of both unmutated and mutated CLL cells, because B-cell...
Receptors of CLL cells are structurally similar to those of antibodies that react with autoantigens and carbohydrate components of infectious agents\(^{41,62,63}\) (Fig. 2B). Alternatively, mutated CLL cells could originate from B cells stimulated in a T-cell–dependent manner that have passed through a germinal center.

Other potential precursors are B1 cells, which share several features with marginal-zone B cells\(^{118}\) as well as immature pre–B cells\(^{119}\) and transitional B cells\(^{120}\) that can also express self-reactive receptors (Fig. 1). A few pre–B cells emerge from the bone marrow into the periphery, and transitional B cells routinely exit the marrow and traverse the
circulation to solid lymphoid tissues. A genetic abnormality could allow one of these cells to survive, thereby making it available for autoantigenic drive and leukemic transformation into unmutated CLL cells.

CLINICAL IMPLICATIONS

PROGNOSIS

The primary role of the Rai and Binet staging systems is to help clinicians decide when patients should be started on therapy. However, since these approaches do not predict the clinical course of a patient with precision, they are less helpful as long-term prognostic indicators. Therefore, physicians have postponed therapeutic decisions until the patients reach advanced Rai or Binet stages. However, the molecular and cellular features we have discussed can distinguish patients with better or worse clinical courses, regardless of the Rai and Binet risk categories. Determination of V-gene mutation is not routinely available, but measurement of ZAP-70 is becoming widely available; it may be the most reliable indicator of prognosis.35

Several points still require clarification and refinement. For example, can a single marker provide a sufficiently accurate and reliable prognostic assessment to permit early decisions about clinical management? Or should several markers be used to increase the degree of accuracy of prognosis? What are the clinically most useful cutoff points for the percentages of expression of CD3826,44 and ZAP-7032-34 and the levels of immunoglobulin V-gene mutation26 that most reliably define the clinical subgroups with various outcomes? Should the cases at the borderlines of these arbitrary cutoffs be handled differently?

MANAGEMENT

In the past, physicians told patients with CLL that a “watchful waiting” mode had to be adopted until the disease progressed, whereupon therapy would be initiated. In my opinion, this approach is especially disturbing, given that the novel prognostic markers indicate that some 50 percent of the patients assigned to watchful waiting have one or more features portending a poor outcome. Although this approach is still being followed, it will probably change considerably when the new prognostic markers are more readily available to all clinicians. On the basis of such information, an early start of therapy may be justified in groups with a poor prognosis. However, before any guidelines can be proposed, the results of large, prospectively conducted clinical trials that test the use of early intervention in patients in poor-prognosis groups must become available. Only one such trial has been initiated, and the accrual of required numbers of patients and the analysis of those results will take several years.

NEW THERAPEUTIC APPROACHES

Since CLL cells must interact with the stroma in bone marrow or other peripheral lymphoid tissues to survive, these interactions need to be explored as targets of innovative therapies. Furthermore, specific inhibition of the B-cell receptor signaling pathway, in particular ZAP-70 or its signaling partners, may be an option. Targeting the actively proliferating cells that maintain the CLL clone by a cell-cycle–active agent could also be considered. Finally, since as many as 20 percent of patients with the worst prognostic markers have stereotypic antigen receptors, these common structures may be practical and valuable points of attack. When the antigens that engage these receptors are precisely defined, it may become possible to develop another arsenal of specific therapies.

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