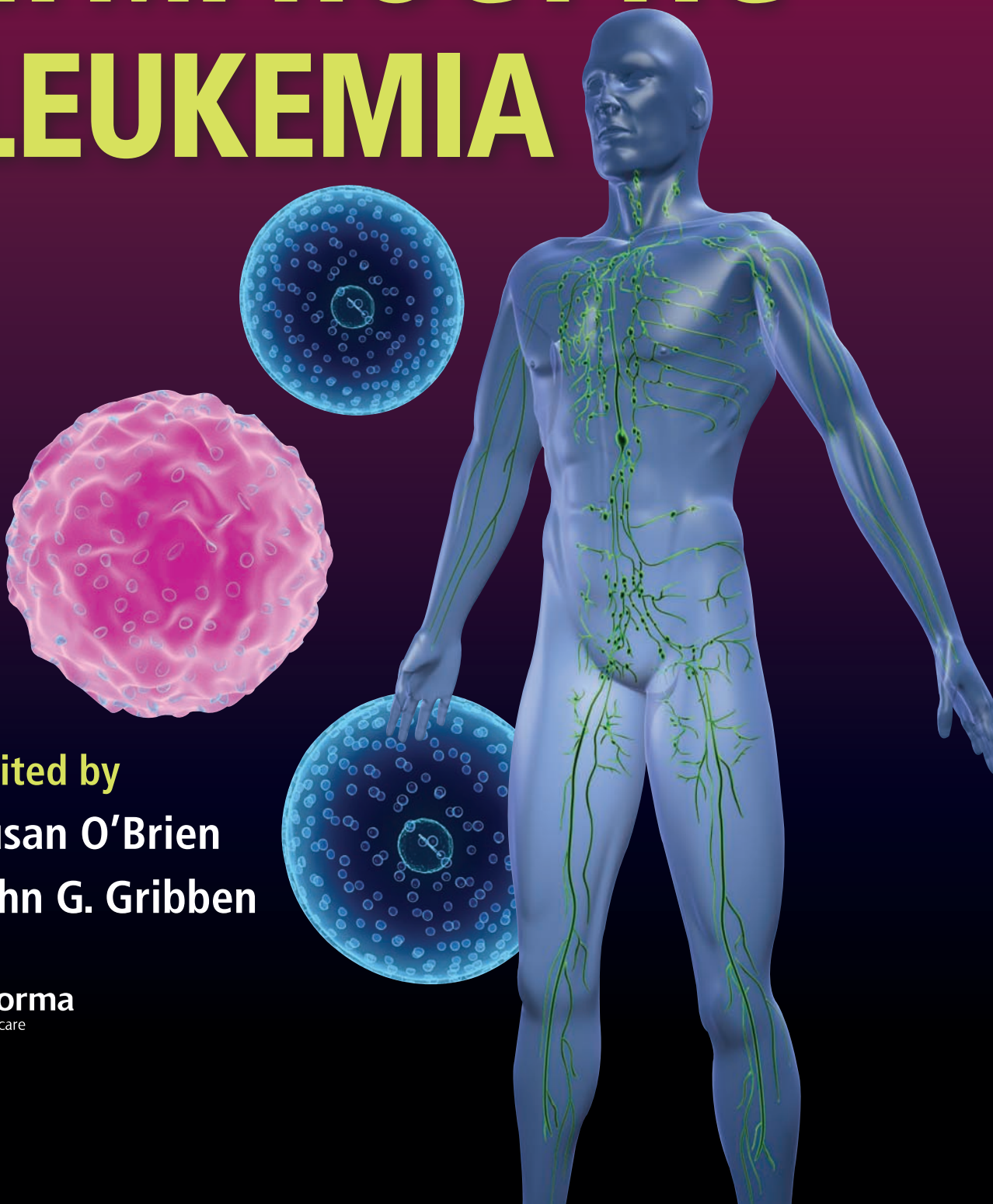
CHRONIC LYMPHOCYTIC LEUKEMIA



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Preface

With this book we have endeavored to develop a comprehensive and up-to-date picture of chronic lymphocytic leukemia. The authors represented herein are some of the leading experts in the field, and the focus is on how new developments in the molecular pathogenesis of this disease impact how we approach and treat patients with CLL. Our introduction to this disease is written by arguably the most famous CLL expert in the United States, Kanti Rai. The initial chapters focus on the origin and nature of the CLL cell and discuss this in relationship to gene expression profiling and molecular abnormalities. Sequencing of immunoglobulin heavy chain genes has shown that patients can be divided into two groups, those with mutated and those with unmutated VH genes (which has significant prognostic import). However, examination of gene expression profiles shows that mutated and unmutated samples are much more similar than different, all having a phenotype of an activated B cell. Thus, these conflicting perspectives on the disease are still being worked out. Another aspect of CLL is the familial clustering that is seen, and the search for genes predisposing to such familial cases is an active area of research. The fascinating finding that a percentage of "normal" people have a small clone of CD5+ B cells provides insight into possible development of the disease, but at the same time raises many questions. Dysregulation of apoptosis is a ubiquitous element in CLL and overexpression of multiple BCL-2 family members can potentially be targeted therapeutically with new molecules that are in clinical trials.

For the past 30 years both the Rai-staging system and the Binet-staging system (which are very similar) have been used to evaluate patients with newly diagnosed CLL. These staging systems are simple, relying only on a physical exam and a complete blood count. Yet they provide significant prognostic information as more advanced stages are associated with shorter survival. However, one limitation of these staging systems is that they are static. That is, in patients presenting with early-stage disease it is difficult to predict which patient is likely to progress and require treatment within a few years and which patient may live 20 years with indolent disease and die of other causes. In the last few years there has been a proliferation of factors, partly derived from research into signaling pathways in CLL, which can provide prognostic information within early-stage disease. Some of these include β2 microglobulin, mutation status of the VH gene, presence of CD38 or ZAP70, and molecular abnormalities detected by fluorescent in situ hybridization (FISH). However, this proliferation of important prognostic factors has also raised the question of correlation between factors, and when discordant, which ones are most important. This is an area under active investigation.

Another potential use of these prognostic factors is identifying a subset of patients who might benefit from early treatment. The approach to CLL has always been a watch-and-wait approach based on a number of features, including the fact that patients tend to be older, with an average age of 70 years, may have very indolent disease and be asymptomatic, and the fact that there is no curative therapy for this disease. Thus, the medical axiom "first do no harm" spares patients who are asymptomatic and have indolent disease from the consequences of therapy that may be unnecessary. However, once a patient requires treatment for CLL their median survival averages seven years. Thus, most patients who require treatment for CLL will eventually die of complications of the disease. These patients can be thought of as "ticking time bombs," where the approach of just watching and waiting (or watching and worrying) does not appear very attractive. The presence of these newer prognostic factors can now clearly identify a population of patients who are not going to survive 10 to 20 years without treatment. However, an important question that is not yet answered is: Does early treatment benefit these patients? It is certainly possible that the same factors that predict for more aggressive disease predict for suboptimal response to the current regimens.

Treatment of CLL has evolved significantly over the past 10 years. Historically, chlorambucil, an oral alkylating agent, was the mainstay of therapy and was an effective palliative treatment with an inability to produce complete remission. Fludarabine, a nucleoside analog, proved to be an effective drug for this disease, and in a randomized trial of fludarabine versus chlorambucil it was shown that fludarabine produced higher complete and overall response rates and significantly longer time to progression. However, overall survival was not impacted. There are several potential reasons for this including the crossover design of the trial, the fact that subsequent therapies may also impact survival, and that the complete remission rate with fludarabine was only 20%. Given that this was the most effective single agent and that upfront treatment is the time when the best response is likely to be obtained, the fact that 80% of patients did not achieve a complete remission is certainly one possibility for lack of impact on survival. This has led to the development of new agents as well as combination regimens, which on early analysis appear to be improving survival in this disease, particular combinations including both fludarabine and the monoclonal antibody rituximab. Another monoclonal antibody, alemtuzumab, was approved for the treatment of fludarabine refractory CLL but may, in fact, be better utilized as a consolidation regimen after debulking by chemotherapy, given that it is exquisitely effective in eradicating marrow disease. Combinations of alemtuzumab and fludarabine are also being investigated.

There are a number of exciting agents in clinical trials, including BCL-2 family member inhibitors, new monoclonal antibodies, HSP-90 inhibitors, cyclin D1 inhibitors, and immunomodulatory drugs. Data relevant to all of these are also discussed in chapter 9.

The advent of nonmyeloablative stem cell transplant has made this modality available for the first time to the majority of patients with CLL. The use of myeloablative transplants typically involved high-dose cyclophosphamide and total-body radiation, which were too toxic for older patients, and so most patients with CLL were not candidates for transplant. Recent trials suggest that long-term survival in CLL can be affected in a proportion of patients who undergo stem cell transplant, and improvements in HLA typing also make this feasible for patients without related donors.

The diagnosis of CLL is accompanied by a number of management issues, including the interesting phenomenon of autoimmunity, the potential for development of Richter's transformation to large-cell lymphoma, and treatment of infections related to disease parameters (cytopenias, hypogammaglobulin), as well as parameters induced by treatment including T-cell deficiencies. All of these are discussed and addressed within the textbook.

We hope that readers will find this book enjoyable to read, highly informative, and at the same time clinically relevant in addressing some of the important questions that are being asked by physicians who have the responsibility of taking care of these patients.

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Introduction

A BRIEF HISTORICAL PERSPECTIVE ON CLL: BENCH VS. BEDSIDE RESEARCH CONTRIBUTIONS

What came first—chicken or the egg? A slight variation of this age-old question can be posed as: Did the progress in our understanding of chronic lymphocytic leukemia (CLL) come first as a result of clinical observations of a few astute physicians or as a result of bench research by a few very smart investigators? Readers might find the chapters that follow in this volume of immense value in answering this question. I would like to start the discussion by citing just a few examples of the dilemma of the primacy issues between bench versus bedside research in this disease.

WHAT IS AT THE CORE OF PATHOPHYSIOLOGY OF CLL? LONG-LIVED LYMPHOCYTES

In the 1960s, Galton (1) and Dameshek (2) came upon their definition of CLL purely by their clinical observations of the natural history of patients with CLL who were under their care. They both suggested that CLL lymphocytes are long lived because they were functionally inert. This was long before the idea of programmed cell death came to the notice of physicians and medical researchers. Even the fact that lymphocytes were broadly classifiable either as B cells or as T cells had not been known when Galton (1) and Dameshek (2) proposed their definition of CLL as a disease of accumulation of long-lived lymphocytes. It took more than a century for basic scientists to find a molecular basis for explaining the longevity of CLL lymphocytes by demonstrating that these cells had altered levels of apoptosis regulating proteins (3) and that these cells had low level expression of miR-15a and miR-16 (4) which, in turn was associated with high levels of bcl-2 (5).

ARE CLL LYMPHOCYTES IMMUNOLOGICALLY NAÏVE?

Until recently, we all believed that CLL lymphocytes are functionally inert and immunologically naïve B cells. This notion was part of the concept of pathogenesis of CLL proposed by Dameshek and Galton. However, recent studies indicate that leukemic lymphocytes in at least half of CLL cases carry mutated IgV_H genes. The process of somatic hypermutation is triggered by a lymphocyte coming in contact with antigen,

which turns a naïve B cell—expressing low-affinity surface Ig into a long-lived memory B cell that is a high-affinity antibody producer (6). These observations not only provided us new insights into the nature of the leukemic B cell in patients with CLL but also enabled us to predict clinical behavior and prognosis in this disease based on whether the patients had somatic mutations in IgV_H genes. These observations demonstrated that not all CLL lymphocytes are immunologically naïve. These bench-based findings, in turn, led to numerous additional subsequent studies (covered in the chapters that follow), which all have had important impact on our ability to assign an accurate long-term prognosis for CLL patients. This is an excellent example of clinical medicine benefiting from basic science research.

SMOULDERING CLL: MONOCLONAL B LYMPHOCYTOSIS OF UNKNOWN SIGNIFICANCE

All clinicians who take care of patients with CLL have long recognized that a small subset of early-stage patients, whose disease was diagnosed purely by chance and whose extent of disease barely fulfills the minimum requirements of diagnosis, seem to have what can be termed "smouldering" CLL (7,8). Such patients have a normal life expectancy without ever showing progression. Recently came startling reports that a small minority of healthy, asymptomatic persons, who may or may not be family members of patients with CLL, carry a tiny number of monoclonal B lymphocytes in their blood that have all the phenotypic markers of CLL (9–11). A question legitimately raised is, Whether such persons can be told that they have a preleukemic phase of CLL? Besides the fact that such news is likely to cause psychological havoc for these persons and their families, it also needs to be emphasized that we have little evidence as to whether all such persons or any of them will develop overt CLL in the course of subsequent years or even decades. Thus, it becomes clear that it will be wrong to accept laboratory findings without simultaneously taking into consideration the clinical picture in every case. Who is to determine whether these findings represent a "pre-smouldering" phase of smouldering CLL or a laboratory research–derived genie escaping the bottle and now becoming a monster for society at large?

The chapters that follow in this volume highlight the progresses made at both the basic science level and the clinical level and provide the reader with a balanced picture of CLL as a disease. There should be no primacy in this chicken-and-egg situation, they both are equally important.

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REFERENCES

1. Galton DAG. The pathogenesis of chronic lymphocytic leukemia. Can Med Assoc J 1966; 94:1005–1010.
2. Dameshek W. Chronic lymphocytic leukemia-accumulative disease of immunologically incompetent lymphocytes. Blood 1967; 29:566–584.

3. Kitada S, Andersen J, Akar S, et al. Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with in vitro and in vivo chemoresponses. Blood 1998; 91:3379–3389.
4. Calin GA, Ferracin M, Cimmino A, et al. A unique microRNA signature associated with prognostic factors and disease progression in B cell chronic lymphocytic leukemia. New Engl J Med 2005; 352:1667–1676.
5. Cimmino A, Calin GA, Fabbri M, et al. mlR-15 and miR-16 induce apoptosis by targeting BCL 2. Proc Natl Acad Sci U S A 2005; 102:13944–13949.
6. Wabl M, Cascalho M, Steinberg C. Hypermutation in antibody affinity maturation. Curr Opin Immunol 1999; 11:186–189.
7. Montserrat E, Vinolas N, Reverter JC, et al. Natural history of chronic lymphocytic leukemia: on the progression and prognosis of early clinical stages. Nouvelle Revue Francaise d'Hematologie 1988; 30:359–361.
8. French Cooperative Group on Chronic Lymphocytic Leukaemia. Natural history of stage A chronic lymphocytic leukemia untreated patients. Br J Haematol 1990; 76:45–47.
9. Rawstron AC, Yuille MR, Fuller J, et al. Inherited predisposition of CLL is detectable as subclinical monoclonal B-lymphocyte expansion. Blood 2002; 100:2289–2290.
10. Rawstron AC, Green MJ, Kuzmicki A, et al. Monoclonal B lymphocytes with the characteristics of "indolent" chronic lymphocytic leukemia are present in 3.5% of adults with normal blood counts. Blood 2002; 100:635–639.
11. Rawstron AC, Bennett FL, O'Connor SJ, et al. Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. N Engl J Med. 2008; 359(6):575–583.

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Origin and Nature of Chronic Lymphocytic Leukemia B Cells

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INTRODUCTION

In the past, B cells were considered a homogeneous population that gave rise to Ig-secreting cells and memory B cells, following specific antigenic stimulation. In recent years, this view has changed, and B cells are now documented as composed of different subpopulations, each with special functions (Fig. 1). These concepts emerged from observations in both humans and experimental animals suggesting that the B cell–rich zone of peripheral lymphoid tissues segregates into functionally unique areas. For example, B-cell proliferation and selection occur in germinal centers (GCs) of lymphoid follicles during an antigenic response, promoting the specific expansion of the cells equipped with B-cell antigen receptors (BCRs) of the highest affinity for the stimulating antigen. In the mantle of lymphoid follicles, there is an accumulation of "virgin" (foreign antigen inexperienced) cells that may be recruited into GCs by antigen stimulation. In contrast, B cells localized in the splenic marginal zone (MZ) can respond in a T cell–independent fashion by producing IgM antibodies against polysaccharide antigens of encapsulated bacteria. B cells with similar features are detected in subepithelial areas of tonsils, subcapsular areas of lymph nodes, and dome regions of Peyer's patches. Cells of lymphoid follicles and those of the MZ have dissimilar phenotypic and trafficking features, mature by distinct pathways, and respond differently to cytokines and chemokines. This further highlights the diversity of B-cell subsets.

Studies in mice have revealed the existence of specialized subsets of B lymphocytes, categorized by functional rather than anatomic criteria. For example, the peritoneal cavity of mice contains a B-cell subpopulation (B-1) that is capable of

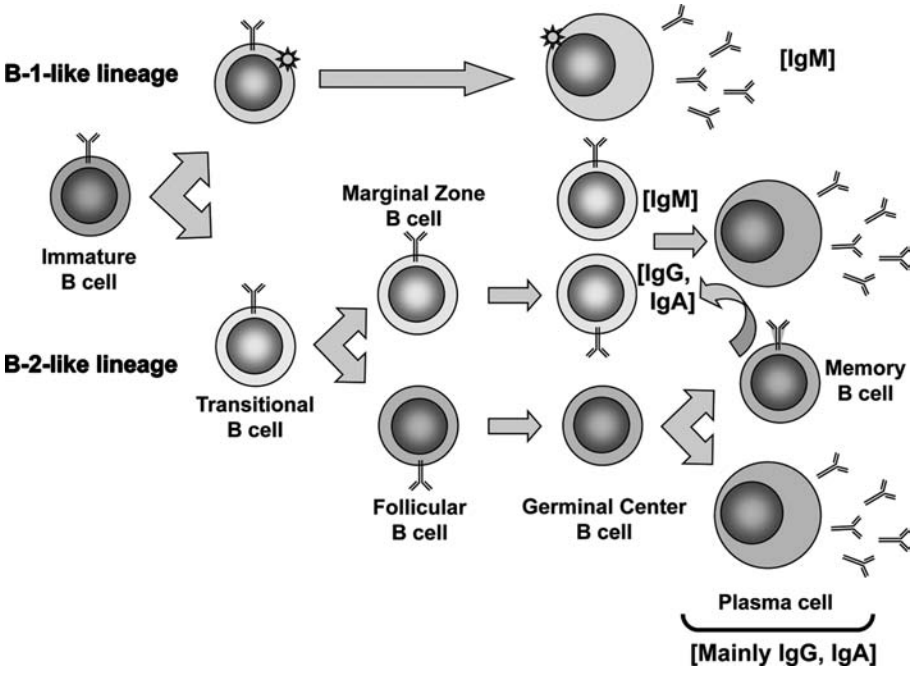

Figure 1 Maturation pathways that normal B lymphocytes follow. This schema is based primarily on studies carried out in mice. The relationship of some of the proposed pathways to human B cells has not been defined (e.g., B-1 cell differentiation pathway) (*See Color Insert*).

self-renewal. These cells produce polyreactive/natural antibodies, mainly of the IgM isotype, reacting with low avidity with a variety of antigens including self- and microbial epitopes. The antigenic determinants recognized by this B-cell subset are frequently nonprotein in nature, consisting of carbohydrates, lipids, and lipoproteins. B-1 cells, which are poorly represented in peripheral lymphoid tissues, are believed to be part of the innate immune system, providing a first line of defense against microbes until an adequate adaptive immune response is achieved. B-1 cells are subdivided into B-1a and B-1b cells on the basis of presence or absence of surface membrane CD5. B-1 cells differ from another functional B-cell subset, B-2 cells, from which high-affinity antibodies and memory B cells specific for stimulating antigens emerge. The phenotypic, trafficking, and maturation features of these B-cell subsets differ markedly.

Human lymphoproliferative disorders, generated by the expansion of a single-cell clone, represent a heterogeneous group of pathological conditions. Part of this heterogeneity is ascribed to their developmental lineages (T cells, B cells, NK cells). The neoplasias emanating from the B-cell lineage are identified on the basis of the B-cell subset of origin. While it has been relatively easy to determine the cell of origin of certain of the B-cell lymphoproliferative disorders (e.g., follicular center cell or MZ lymphomas), for others the cell of origin is still a matter of debate. This is the case for chronic lymphocytic leukemia (CLL), and the issue is especially complicated by the divergent molecular features of cells from patients that differ in clinical course. Although a definite answer to the question of the origin of CLL cells is presently unavailable, we shall review here the principal phenotypic, genetic, structural, and functional features of CLL cells and compare these with those of the major known B-cell subsets.

DISTINGUISHING CHARACTERISTICS OF CLL CELLS THAT COULD PROVIDE CLUES TO THE NORMAL B-CELL EQUIVALENT

As mentioned, B cells are divided into subsets on the basis of several criteria, including expression of cell surface molecules, location in specific geographic regions of lymphoid organs, use of specific genes, and functional properties. Applying these to CLL cells, characteristic patterns emerge.

In this analysis, we shall take into account that there are two major subgroups of CLL, characterized by the use of unmutated versus mutated immunoglobulin heavy chain variable (IgV_H) gene segments (1); patients in the former group (U-CLL) have a more aggressive clinical course with shorter survival than patients in the latter group (M-CLL) with a more indolent course and longer life span (2,3). In general, the cells from U-CLL also express ZAP-70 and CD38, while M-CLL cells do not (2,4–8). There are also other distinguishing features between the cells from the two CLL subsets that will be described below.

Surface Membrane Phenotype

CLL cells express surface membrane CD5, along with CD23 and CD27 (9,10). Other distinguishing phenotypic features include diminished levels of surface membrane Ig and CD22. The $CD5^+CD23^+CD27^+smIg^{low}$ phenotype is generally consistent in CLL, although the percentage of cells within a given clone expressing individual molecules can vary. FMC7 and CD10 are usually not displayed on CLL cells, and therefore their expression can be used to distinguish CLL from other types of leukemia should they be present on large numbers of the leukemic clone (e.g., hairy cell leukemia).

Although the expression of a common surface membrane phenotype by CLL cells from multiple patients suggests that the two commonly delineated subgroups of CLL (U-CLL and M-CLL) may derive from the same normal B-cell precursors, the leukemic cells of these two subsets of patients do differ in expression of "activation markers." U-CLL more often than M-CLL express ZAP-70 and CD38 (2,4,5), both activation markers for normal B cells (11–14). In addition, the differential expression of additional molecules upregulated by cell activation (e.g., HLA-DR, CD69, CD71, CD62L, and others) suggests a more marked and recent activation of U-CLL cells (15).

Unfortunately, expression of several of the molecules that define the phenotype of CLL and its subtypes can change at different stages of maturation and activation of normal human B cells (e.g., CD5 and CD38). Furthermore, the defined surface membrane expression pattern of CLL cells does not correlate with a specific normal human B-cell subset. Therefore, the use of surface membrane phenotype alone to assign a CLL cell to a specific normal human B-cell subset can be treacherous.

Anatomic Location and Pattern of Growth

CLL cells circulate throughout the body via blood and lymph vessels, orchestrated by a series of chemokine receptors—CXCR3, CXCR4, CXCR5, and CXCR7—that are functional based on in vitro studies (16). In particular, CXCR4 allows CLL cells to sense a chemokine gradient of its ligand CXCL12/SDF-1, guiding CLL cells to stromal cells producing the ligand (17–19). Upon engagement with CXCL12/SDF-1, CLL cells migrate beneath the stromal cells via a process termed pseudoemperipolesis (17). CXCR3 is also relatively well expressed on CLL cells, in contrast to normal circulating human B cells