B-Cell Chronic Lymphocytic Leukemia: Present Status and Future Directions

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CLL EPIDEMIOLOGY

CLL constitutes a varying proportion of all adult leukemias, ranging from 2.5% in Japan to 38% in Denmark. In most Western countries, irrespective of sex and race, CLL is the leukemia type with highest incidence among persons aged 50 to 55 years and older, and its rates increase with age. This pattern differs in West Africa, where low CLL rates are observed within two broad categories: women aged 40 to 44 years and males aged 70 years and over. Finally, there are some geographic variations within countries, with the highest incidences in rural areas.

The relationship between CLL and socioeconomic status remains unclear. Nevertheless, CLL has been linked with several occupations and exposures. An increased risk of CLL has been reported among farmers with high levels of soybean production, cattle raising, dairy production and herbicide use, among rubber manufacturing workers, and tire repair workers. Finally, CLL is the only leukemia type that has not been related with occupational exposure to radiation.

Some of the ethnic and racial variations could be explained by genetic factors. Familial aggregation of CLL has been reported, with excess in first-degree relatives of CLL ranging from twofold to sevenfold compared with controls. An underlying inherited chromosome abnormality (eg, of chromosome 12) or a high frequency of chromatid exchange figures (eg, 14q+) could predispose to CLL, as well as consanguinity. Few associations with HLA antigen have been identified. The possible effect of chronic infections, connective tissue disorders, or allergic conditions in CLL remains controversial.

CLINICAL AND LABORATORY FINDINGS

Clinical findings in CLL are generally limited to blood lymphocytosis, with or without peripheral lymphadenopathy and/or hepatosplenomegaly. Diagnosis of CLL is more and more often made at an early stage of the disease, patients with peripheral blood (PB) and marrow lymphocytosis, without organomegaly or cytopenias (Rai's stage 0) accounted for 29% of our 973 patients diagnosed between 1980 and 1985 as compared with 39% of the 1,279 patients diagnosed between 1985 and 1990.

Although involvement of any other organ system is possible in CLL, including skin, gastrointestinal tract, lungs and pleura, central nervous system or peripheral nerves, kidneys, bone, and salivary and lachrymal glands, it is rare, and should be distinguished from infection, adverse effects of treatment, other neoplasia, and progression to a more aggressive lymphoproliferative disorder.

Minimum requirements for a diagnosis of CLL include sustained lymphocytosis of greater than 10x10^9/L in PB with mature appearing lymphocytes and bone marrow (BM) lymphocytosis of at least 30% in BM aspirates. In patients with 5 to 10x10^9/L lymphocytes, the permanent nature of the lymphocytosis should be documented, and cell marker studies like CD5 expression by B cells and/or clonal excess of membrane \(\kappa\) and \(\lambda\) chain are necessary to establish clonality. Although blood lymphocyte counts may be as high as 1,000x10^9/L, about two-thirds of the patients now present with lymphocytes less than 30x10^9/L. In typical cases of CLL, the cells are small with a narrow rim of cytoplasm, and the nuclear chromatin is dense and nucleoli are not visible. However, in some cases of CLL, morphologic features at diagnosis are different, including (1) a spectrum of small to large lymphocytes with narrow rim of cytoplasm, and the nuclear chromatin is dense and nucleoli are not visible. However, in some cases of CLL, morphologic features at diagnosis are different, including (1) a spectrum of small to large lymphocytes with occasional (less than 10%) prolymphocytes and (2) a...
mixture of small lymphocytes and prolymphocytes (>10% and <55%) designated CLL/PL.36 Those two variants have been described as CLL, mixed cell types.35,36

Anemia and thrombocytopenia are now relatively rarely found at diagnosis and mainly occur during the course of the disease (see below). Four different patterns of lymphoid infiltration are usually recognized on BM biopsies72,38: nodular, interstitial, mixed (nodular and interstitial), and diffuse; but sequential studies show that they represent a dynamic process, with progression to diffuse infiltration occurring coincidentally with clinical progression.39

CLINICAL COURSE

Although very rare cases of spontaneous remission in CLL have been reported39 and a significant proportion of patients remain stable for years, the total tumor cell burden in CLL generally tends to expand at a variable speed. The most frequent complications include infection, blood cytopenias, and transformation of the disease.

Infections occur mainly at advanced stages.40 Susceptibility to infection results mainly from hypogammaglobulinemia, but also from specific antibody deficiency, depleted cell-mediated immunity, neutropenia, and poor opsonization. Infections are mainly bacterial and viral infections (generally due to herpes viruses: herpes simplex or zoster); mycobacterial and fungal infections are less frequent.

Anemia in CLL may result from immune hemolysis (see below), excessive spleen pooling, reduced red blood cell (RBC) production associated to massive BM infiltration, or pure RBC aplasia, of probable immune origin.41 Those mechanisms, however, may be associated. Thrombocytopenia may also result from decreased platelet production by infiltrated BM and/or by platelet destruction by platelet autoantibodies42 or excessive spleen pooling in cases with prominent splenomegaly. Immune neutropenia is rarely seen and is a feature of T- rather than B-CLL.43

In a small proportion of cases, CLL may evolve to more aggressive forms, mainly by develop of diffuse large cell lymphoma (Richter’s syndrome).44 Richter’s syndrome, which develops in 3% to 10% of CLL is heralded by sudden onset of general symptoms or a rapidly progressive tumor mass whose biopsy shows large cell immunoblastic lymphoma along with evidence of CLL in sites not involved by lymphoma. Response to chemotherapy is generally poor, and median survival is only 4 months. Other types of transformation include: (1) “prolymphocytoid” transformation, with growing numbers of prolymphocytes in blood samples, and increasing tumor mass, cytopenias, and resistance to treatment45 (It remains uncertain, however, whether this entity differs or not from CLL/PL); (2) acute lymphoblastic leukemia (ALL), of which only a few cases have been clearly documented,46 and which has to be distinguished from exceptional cases of acute myeloid leukemia (AML), in patients who had generally received prolonged treatment with alkylating agents; and (3) myeloma, which is also exceptional in CLL.37 Whether Richter’s syndrome and other transformations represent clonal evolution of CLL or are independent disorders remains controversial.44

Finally, CLL patients seem to have an increased risk of nonhematologic neoplasms as compared with control patients or patients with other malignancies.47,48 However, The French Cooperative Group on CLL (FCG on CLL) recently reported that the increase in epithelial tumors was mainly found in patients who had received long-term continuous chlorambucil,49 and it is uncertain whether untreated patients also have an increased risk of other neoplasms.

DIFFERENTIAL DIAGNOSIS

CLL is generally easy to distinguish from reactive lymphocytosis. The following mature B-cell malignancies should be distinguished from B-CLL on the basis of cytology and membrane phenotype: (1) B-prolymphocytic leukemia (PLL),50 characterized by extreme leukocytosis (>100 × 109/L) and splenomegaly, with minimal or no lymphadenopathy. The prolymphocyte is the predominant cell in PB (>55%, usually >70%) and is characterized by its large size, prominent nucleolus, and lower nuclear:cytoplasmic ratio than the small CLL lymphocyte. The membrane phenotype is different from that of CLL, including increased expression of surface Igs, low mouse rosette formation, low CD5 expression, and positivity for FMC7. As seen above, however, CLL/PL has features intermediate with those of classic CLL and classic PLL, and includes patients with typical CLL that evolves into “prolymphocytoid” transformation, as well as patients with an increased proportion of prolymphocytes at diagnosis. (2) Hairy cell leukemia (HCL), which may be confused with CLL in those rare forms with leukocytosis and a high percentage of circulating hairy cells (so called HCL “variant,” which has morphologic features intermediate between those of hairy cells and prolymphocytes).51 Diagnosis of HCL, however, can usually be made by morphologic, tartrate-resistant acid phosphatase positivity, and the combination of CD25, LeuM5, and HC2 positivity. (3) Splenic lymphoma with circulating villous lymphocytes (SLVL),52 associated with massive enlargement of the spleen, moderate hyperleukocytosis (10 to 30 × 109/L), small monoclonal bands in serum or urine in 60% of cases, circulating lymphocytes characterized by cytoplasmic short villi, and membrane markers similar to those of B-PLL. (4) Leukemic phase of non-Hodgkin’s lymphoma (“lymphosarcoma cell leukemia”), generally of follicular or diffuse small cleaved cell histology.53,54 Typical cells are often pleomorphic with nuclear clefting. The disease can also be distinguished from CLL by lymph node biopsy, histologic BM pattern, and immunologic features (strong surface Igs, low percentage of mouse rosettes, positivity of FMC7 and, often, of CD10, negativity of CD5). (5) Waldenström’s macroglobulinemia, in cases where circulating lymphocytosis is present. The malignant lymphocytes generally have plasmacytoid features, however, and secrete substantial monoclonal IgM in the serum.

B-CLL should also be differentiated from mature T-cell disorders, which are far less frequent than mature B-cell disorders, at least in Western countries, and have been classified into four main types, including T-CLL (or large granular lymphocyte leukemia), T prolymphocytic leuke-
mia, adult T-cell leukemia/lymphoma, and Sezary syndrome.

PROGNOSTIC FACTORS

Clinical Staging Systems

Although some prognostic factors, including sex, age, peripheral lymphocytosis, lymph node and spleen enlargement, anemia, and thrombocytopenia, had been reported for some time in CLL, the specific importance of each of these factors remained uncertain until the staging proposals of Rai et al were published in 1975.24

The clinical staging of Rai et al24 segregates CLL patients into five groups: stage 0, lymphocytosis in blood and BM only; stage I, lymphocytosis and enlarged lymph nodes; stage II, lymphocytosis plus hepatomegaly, or splenomegaly, or both (nodes may or may not be enlarged); stage III, lymphocytosis and anemia (hemoglobin [Hb] < 11 g/dL) (nodes, spleen or liver may or may not be enlarged); and stage IV, lymphocytosis and thrombocytopenia (platelets < 100 x 10^9/L) (anemia and organomegaly may or may not be present). The prognostic value of this staging system was validated by several investigators.25-27 Recently, Rai et al suggested that there were essentially three rather than five groups that differed with respect to survival: stage 0 (good prognosis), stages I and II (intermediate prognosis), and stages III and IV (poor prognosis).

The large number of groups in Rai’s system and the availability of new statistical methods such as the Cox model led Binet et al28 to define a new staging system in 1981 that includes three groups: stage A, with less than three areas involved (areas include the cervical, axillary, and inguinal lymph nodes, whether unilateral or bilateral spleen and liver), Hb >= 10 g/dL, and platelets >= 100 x 10^9/L; stage B, with at least three areas involved, Hb >= 10 g/dL, and platelets >= 100 x 10^9/L; and stage C, Hb less than 10 g/dL, or platelets less than 100 x 10^9/L or both (independently of the areas involved). The validity of this system was confirmed in six retrospective series and in a prospective series of 973 cases.29

Comparison of both systems in French series shows that Binet’s good prognosis group (stage 0) includes 30% of all patients with a 7-year survival of 76%, whereas Binet’s good prognosis group (stage A) includes 63% of CLL patients, with a 7-year survival of 67%.30 Two-thirds of Rai’s stage I and one-third of Rai’s stage II (considered of intermediate prognosis) are also included in Binet’s stage A. From a therapeutic point of view, this result may constitute an advantage of the A, B, C system because there is a general consensus that stage A patients should not be treated unless they progress.27,33,40 The intermediate prognostic group (stage B) in Binet’s system includes 30% of patients who have a poorer prognosis (median, 57 months) than the intermediate group from Rai (stages I and II), which includes 59% of patients with a median of 83 months. Finally, because the cut-off value for Hb is 10 g/dL in Binet’s staging, fewer patients are included in the high-risk group (7% for stage C) as compared with 11% for Rai’s staging (11% for stages III + IV).

Clinical staging systems other than those of Rai and Binet have been proposed in CLL,25,33,54 but they have not been used extensively in clinical practice. The International Workshop on CLL (IWCLL)35 recommended the adoption of an integrated Binet-Rai staging system, in which the Binet stage (A, B, C) was to be further defined by adding the appropriate Rai stage (0, I, II, III, or IV).

Other Prognostic Factors

Three parameters have shown independent prognostic value in CLL in addition to clinical staging: rapid lymphocyte count doubling time (less than 12 months) is associated with short survival36; a diffuse pattern of infiltration carries a poor prognosis, whereas all others (ie, nondonfuse) patterns give a better survival37,38; and patients with a normal karyotype fare better than those with an abnormal one.35-37 Among the latter, patients with single abnormalities have longer survival than patients with complex karyotypes. Although, some reports suggested that trisomy 12, when isolated, did not carry a poor prognosis,38 a cooperative study on 433 cases of CLL found, on the contrary, a shorter survival in patients with trisomy 12, even when isolated.39 In this study, patients with single abnormalities involving chromosome 14q also had shorter survival than those with single aberrations of chromosome 13q and, possibly, 6q, who had the same survival as those with a normal karyotype.39

Increasing age is associated with shorter survival, although the difference loses part of its significance when non-CLL deaths are removed. A higher incidence of more aggressive forms (stages B and C) has even been found in younger patients in some series32 and in our experience (unpublished). Shorter survival is also seen in males, even after excluding non-CLL deaths.32 Circulating lymphocyte count is correlated with survival and threshold values of 30 to 60 x 10^9/L help identify good and poor prognostic subgroups.21-24

The prognostic value of other prognostic factors, including surface Ig isotype,56 low serum Ig levels,56 high serum deoxothyridine kinase levels,60 high serum levels of soluble CD23,61 and high CR2/CR1 complement receptors, remains controversial.62

Smouldering CLL

Both Rai’s and Binet’s staging systems are limited in that they are unable to distinguish patients in the low-risk group who will have an indolent course and good prognosis from a small fraction who will progress. Some of the prognostic variables described above, in addition to threshold values for certain clinical and hematologic parameters, may be useful in such a situation.

Montserrat et al35 recently showed that Binet’s stage A patients with a lymphocyte count of less than 30 x 10^9/L, a lymphocyte doubling time of greater than 12 months, Hb greater than 13 g/dL, and nondonfuse BM histology were very unlikely to progress and had a life expectancy that was not different from that of the sex- and age-matched population. They suggested that these patients should be termed “smouldering” CLL. After 3 years, only 5% of these
patients showed disease progression, as compared with 52% of the other stage A patients. Likewise, the FCG was able to divide 309 untreated stage A patients into (1) stage A’, characterized by a lymphocyte count less than 30 × 10^9/L and Hb greater than 12 g/dL (80% of stage A patients); and (2) stage A” with either a lymphocyte count greater than 30 × 10^9/L or Hb less than 12 g/dL (20% of stage A patients). Five-year survival was 87% in stage A’ and did not differ from that of an age- and sex-matched French population and was 60% in stage A”. Moreover, survival in stage A’ was identical to that of the group defined by Monserrat et al. The progression rate at 5 years was 25% and 54%, respectively, in stages A’ and A”. Most other groups have confirmed the prognostic value of Hb, lymphocyte count, lymphocyte doubling time, and BM pattern in survival and/or disease progression in patients with Binet’s stage A. Progression from stage A was correlated with Rai substage in at least two studies, and with the presence of a complex karyotype in the Bourne- mouth group. A group of 20 patients with Rai stage 0, normal karyotype, and stable disease for 6.5 to 24 years had also been previously reported, and was designated as having “benign monoclonal B-cell lymphocytosis.”

Although the choice between competing proposals remains somewhat arbitrary, a definition of smouldering CLL appears feasible. Whatever definition is used, survival rates of patients with smouldering CLL are similar to those of the general population. However, a small proportion (about 10%) of them will progress to stage C within 5 years and criteria are not currently available to initially detect them. Widespread use of BM biopsy, cytogenetic analysis, and other biologic studies may help to more precisely define smouldering CLL.

**BIOLOGY OF B-CLL**

### Phenotypic Characteristics of CLL B Lymphocytes

CLL cells express surface membrane Ig (SmIg), C3dr complement receptors, and receptors for the Fc fraction of Ig. SmIg are constantly restricted to a single light chain and frequently express IgM or both IgM and IgD. In the latter case, IgM and IgD share idiotypic determinants, thus attesting to the monoclonal origin of the cells. This monoclonal origin has been further confirmed by the presence of a single pattern of glucose-6-phosphate dehydrogenase, clonal chromosomal abnormalities, and the presence of unique Ig gene rearrangements.

CLL B cells also express several antigens, including D-related human leukocyte antigens and antigens related to B cells. Most cases of B-CLL appear to react with CD19, CD20, CD24, CD37, and CD21 monoclonal antibodies (MoAbs). About 60% of CLL are positive for CD23, whereas membrane positivity with CD22 is infrequent. CLL B cells and HCL B cells have been found to express a 69-Kd glycoprotein that is not expressed by normal blood T and B lymphocytes, thymocyte-cultured T- and B-cell lymphoblastoid cell lines, or acute lymphoblastic leukemia cells. In contrast to B-cell prolymphocytic leukemia, which constantly binds the FMC7 antibody, the reactivity of CLL B cells is not frequent. CALLA (CD10) is almost constantly negative, whereas reactivities with subepitopes of CD1, and CD11, as well as with CD6, CD7, and the TQ1 antigen, have been reported in some cases of B-CLL. Interestingly, myelomonocytic antigens have also been found to be expressed by CLL B cells.

Contrary to most other B-cell malignancies, CLL B lymphocytes are characterized by three particular phenotypic patterns: (1) B-CLL lymphocytes almost always express low amounts of SmIg, although increased amounts of intracytoplasmic Ig have been observed; (2) CLL-B lymphocytes, 31% to 95% frequently form rosettes with mouse erythrocytes; and (3) in most cases, they express the CD5 antigen, a 67-Kd antigenic determinant initially described as a pan-T-cell marker. The normal counterpart of the CD5 B cells that proliferate in CLL was initially found by Caligaris-Capio et al. Furthermore, a substantial number of B cells in 20-week-old fetal lymph nodes and spleen express the CD5 marker as well as μ and δ chains on their membrane. These fetal cells also appeared to share lectin nonresponsiveness and the inability to cap SmIg with CLL B lymphocytes, supporting the idea that they were normal counterparts of B-CLL lymphocytes. With the advent of double-labeling cytofluorometry techniques, it is presently clear that about 15% of normal B cells express CD5 markers.

Morphologically, CLL B lymphocytes resemble small resting B cells. However, during recent years, considerable evidence has accumulated indicating that CLL B cells frequently express activation antigens such as CD23; CD25, B5, and blast 1, whereas BB1 and CD71 are rarely observed. A high prevalence of CD5 cells during early ontogeny led some investigators to assume that the CD5+ B-CLL B lymphocyte corresponds to expansion of an immature B-cell clone arrested at an immature stage between pro-B and mature B cell. However, this hypothesis does not provide a satisfactory explanation for the difficulty of B-cell differentiation pathways to integrate CD5 antigen expression, not for the high frequency of hypogammaglobulinemia and autoimmunity directed against blood cell components in B-CLL. The latter phenomena are only rarely observed in other B-cell neoplasias. Alternatively, it has been postulated that CD5+ B cells could correspond to a separate B-cell lineage. There is some evidence indicating that Ly1 B cells, the murine counterpart of human CD5 B cells, constitute a discrete B-cell subset. This evidence results from experiments on lethally irradiated mice and from the observation of different behaviors in long-term cultures. However, no definitive evidence indicating that CD5+ B cells constitute a different B-cell lineage is presently available.

### B-Cell Differentiation, Antibody Activity, and Gene Expression

Although earlier studies postulated that B-CLL lymphocytes were frozen at an early stage of differentiation, considerable evidence has accumulated that these cells are able to differentiate. Stevenson et al. using idiotypic reagents, have shown that the pentameric form of SmIg was...
present in CLL serum. Some studies have indicated that the heavy chain switch can occur in B-CLL, and hence, some investigators have suggested that there is a certain degree of maturation of malignant cells. In vitro experiments with different mitogens, such as pokeweed, nocardia, or phorbol esters, have succeeded in inducing differentiation of CLL B lymphocytes. With phorbol esters, the increased presence of RNA coding for secretory IgM was observed. Interestingly, on phorbol ester stimulation, normal CD5- B cells expressed CD5 markers, and B-CLL cells developed tartrate-resistant phosphatase activity and resembled hairy cells, whereas with lipopoly saccharide (LPS), they were found to express increased levels of SmIg and the FMC7 marker. A variable pattern in the response to different cytokines such as interleukin-2 (IL-2), B-cell growth factors (BCGF; interferon α [IFNα] and IFNγ and IL-4) has been reported in B-CLL; this may be indicative of discrete stages of maturation and activation in B-CLL patients. Among these cytokines, IL-2 appears to be the most consistent activator, whereas IL-4 downregulates CLL B lymphocytes, in contrast to its effect on normal B cells.

The antibody activity of CLL was only recently assessed by studying the antibody activity of Ig-containing supernatants obtained after stimulation of CLL B lymphocytes with phorbol esters, or by studying the antibody activity of hybridomas derived from CLL B lymphocytes. All reports succeeded in showing a high proportion of CLL B cells displaying natural autoantibody activity. Indeed, about half of the CLL B cells displayed rheumatoid factor activity and about 20% showed multispecific activity against autoantigens such as DNA and cytoskeleton proteins.

In a recent work, Kipps et al. found that a high proportion of B-CLL cells expressing κ at the membrane reacted with a murine antiidiotype antibody raised against a monoclonal IgM rheumatoid factor expressing the Wa idiotype (major cross-reactive idiotype expressed by cryoglobulins). Analysis of κ light chain variable region genes expressed by leukemic cells from different patients sharing the Wa idiotype enabled these investigators to show that they all used the unmutated germinal Hum Kv 325 gene. Similar restriction was found when VH genes were analyzed. These results confirm that CD5- B-CLL lymphocytes are frequently committed to the production of natural autoantibodies. Furthermore, results from Kipps et al. strongly suggest the use of a restricted set of genes by CLL B lymphocytes and this was confirmed by the high frequency of natural autoantibody activity found among CLL B lymphocytes. Because CD5- B lymphocytes from follicular non-Hodgkin's lymphomas have also been found to be frequently committed to secretion of natural autoantibodies, these results appear to indicate that the autoreactive B-cell repertoire is frequently involved in malignant transformation.

T and Natural Killer (NK) Cells in B-CLL

In B-CLL, a significant increase in T and NK cells has been found in untreated patients. Decreased T-helper cells, increased T-cytotoxic/suppressor cells, and inversion of the T4/T8 ratio have been reported. Decreased helper T-cell function has been frequently reported, whereas some studies suggested increased suppressor function. The expansion of these cells is probably polyclonal in nature, as indicated by the lack of chromosome abnormalities and heterozygosity for glucose 6-phosphate dehydrogenase. The reason for this polyclonal expansion of T and NK cells in B-CLL is unknown. However, a recent work claimed that 25% of B-CLL displayed clonal rearrangements of the T-cell receptor β from T cells. Whether these unexpected results favor the idea that in some cases the CLL target cell could correspond to a stem cell with both B- and T-cell differentiation ability and in some cases with myeloid differentiation potentialities (as attested by occasional expression of these markers) is as yet unclear. Functional studies of T cells from B-CLL patients indicate that mitogenic responses to phytohemagglutinin (PHA) are usually but not always normal, whereas reactivity to autologous or allogeneic B cells is impaired. Data concerning helper, suppressive, NK, and ADCC function are contradictory and difficult to interpret.

Hypogammaglobulinemia in B-CLL

Hypogammaglobulinemia occurs in 10% to 60% of B-CLL cases, depending on the values used as the lower limit. Patients with early forms of the disease tend to have defective specific antibody responses to infection or immunization. Hypogammaglobulinemia is probably a consequence of accumulation of these individual defects. The pathogenesis of hypogammaglobulinemia in B-CLL is poorly understood, because this phenomenon is rare in other B-cell malignancies including ALL, nodular and diffuse lymphomas, HCL, and prolymphocytic leukemia, although it is common in multiple myeloma. Although regulatory abnormalities in T cells may play a role in the induction of hypogammaglobulinemia, data concerning helper, suppressive, NK, and ADCC are contradictory, and fail to firmly establish their contribution to the development of hypogammaglobulinemia. Based on the information presently available, it appears logical to assume that hypogammaglobulinemia in B-CLL is probably the result of dysfunction of nonclonal B cells. Thus, hypogammaglobulinemia in B-CLL could be a consequence of progressive dilution or inhibition of normal CD5- B cells. This decrease and/or inhibition of normal CD5- B cells could also explain the classical inability of B-CLL to respond to new antigenic challenges, because Ly1 B cells (the murine counterpart of human CD5 B cells) have been claimed to be unable to respond to exogenous antigens. A prominent monoclonal Ig peak, usually of the IgM type, is found in 5% of CLL. With high-resolution agarose gel electrophoresis and immunofixation, however, a small amount of a monoclonal component can be identified in the serum or urine of 60% of patients.

Autoimmune Phenomena in B-CLL

Autoimmune-associated phenomena are frequently observed in B-CLL. These autotoxic manifestations are mainly
directed against hematopoietic cells.\textsuperscript{136} A positive direct antoglobulin test has been reported to be as high as 7.7% to 35% of B-CLL patients, depending on the series and stage of disease.\textsuperscript{136} In the FCG series (CLL 1980 and 1985), positive Coombs test was found at diagnosis in only 1.0%. This lower prevalence is explained probably by the fact that the FCG series included higher numbers of initial forms than previous series. Nevertheless, autoimmune hemolytic anemia occurs in 10% to 25% of patients at some time during the course of the disease. Although Feizi et al\textsuperscript{137} described one case of cold agglutinin disease in which autoantibodies were the product of CLL B cells, in most cases autoantibodies against RBCs are warm reactive polyclonal IgG and display activity against monomorphic antigens of the rhesus system.\textsuperscript{138} Immune thrombocytopenia is observed in about 2% of cases, but higher frequencies of increased platelet associated Igs have been reported.\textsuperscript{37,138} Pure RBC aplasia and autoantibodies against neutrophils are only rarely observed and there is conflicting evidence concerning the frequency of other autoantibodies.\textsuperscript{139} This pattern is similar to that observed in primary immunodeficiency syndromes, in which immune thrombocytopenia, autoimmune hemolytic anemia, and pure RBC aplasia are frequently observed.\textsuperscript{140} Because in B-CLL autoantibodies in most cases are not secreted by the malignant clone, it can be postulated that hypogammaglobulinemia could induce a disturbance in the idiotypic network, in such a way that anti-idiotypic antibodies designed to antagonize autoimmune clones are not made.\textsuperscript{141} Recently, Sultan et al\textsuperscript{142} succeeded in suppressing production of anti-VIII autoantibodies by injecting intravenous Ig and speculated that anti-idiotypic suppression mediated by injected IgGs could occur. However, no conclusive evidence concerning the role of intravenous Ig in the treatment of autoimmune-associated phenomena in B-CLL have been reported so far.

**Chromosomes and Oncogenes**

In recent years, the use of mitogens, such as Epstein-Barr virus, phorbol esters, LPS, and pokeweed, has made it possible to obtain sufficient metaphases for analysis in most cases of CLL. In more than 50% of patients, chromosomal abnormalities are detected. The most frequent abnormality in B-CLL, found in one-third of the abnormal cases, is trisomy 12.\textsuperscript{155-157} In a large cooperative study,\textsuperscript{56} the most frequent structural aberration (51 of 433) affected the 13q14 band, in which the retinoblastoma tumor suppressor gene has been mapped. 14q 32 band rearrangements were found in 41 patients. Less frequently, alterations of chromosomes 11, 6, 18, 3, 17, 7, and 8 were observed.\textsuperscript{146} In most cases, chromosomal abnormalities remained unchanged throughout the disease.\textsuperscript{146} However, this absence of clonal evolution during progression of the disease will have to be confirmed, because it is in contrast with what is observed in other chronic hematologic malignancies (especially, chronic myeloid leukemia or myelodysplastic syndromes).

Little data on a role for proto-oncogene activation in the pathogenesis of CLL are currently available. Most proto-oncogenes have been found to be normally expressed in B-CLL.\textsuperscript{146} However, Oscier et al recently found a deletion of the retinoblastoma gene in several patients with B-CLL and a rearrangement involving band 13q 14.\textsuperscript{141} A recent study of 32 CLL patients showed bcl-2 rearrangements in three patients, resulting from the juxtaposition of bcl-2 and one of the Ig light chain genes.\textsuperscript{142} In all cases analyzed, the breakpoints were observed at the 5' flanking region of the bcl-2 gene, and did not involve the major or minor breakpoint cluster region typical of the t(14:18) chromosomal translocation observed in follicular lymphomas.\textsuperscript{143,144} Rare cases of CLL with (11:14) (q13:q32) translocation involving the J region of the Ig heavy chain and a putative protooncogene (bcl-1) located on chromosome 11 have been reported.\textsuperscript{145} However, this translocation is more frequently observed in lymphocytic lymphoma of intermediate differentiation\textsuperscript{146} and in some cases of B-PLL\textsuperscript{148} than in true B-CLL.\textsuperscript{146} Finally, a 14:19 translocation involving the class switch region genes of the α-heavy chain constant region and a putative proto-oncogene located on chromosome 19 (bcl-3) has also been reported.\textsuperscript{149,150} Those rearrangements, however, which affected the Ig heavy and light chain genes, were observed in a minority of CLL.

**Therapy in CLL**

The variable course of CLL, the advanced age of most of the patients, and the absence of uniform response criteria have constituted important setbacks in the therapy. Thus, an analysis of therapeutic results obtained before the advent of staging systems was almost impossible. Stage A patients, Rai's stage 0 patients, and Rai's I and II allocated into Binet's stage A (because their prognosis is close to that of Rai's stage I) should not be treated unless they progress. On the contrary, there is a general agreement that stage B and C patients should be treated.\textsuperscript{53,54} With the aim of standardizing the response criteria to treatment, the IWCLL and the National Cancer Institute (NCI) Sponsored Working Group defined complete remission (CR), partial response (PR), stable disease (SD), and progressive disease (PD), as shown in Table 1.\textsuperscript{155,156} In fact, all of these criteria define clinical remission rather than CR, which seems very difficult to obtain. Furthermore, assessment of CR is difficult to make with current therapeutic approaches. More sensitive methods are required to detect residual malignant cells. Flow cytometry with the simultaneous use of CD5 and CD19 or CD20 markers, \(\times\) clonal excess, and analysis of gene rearrangements by the polymerase chain reaction are under study in this setting.\textsuperscript{151,152}

**Single-Agent Chemotherapy**

Chlorambucil (CLB) remains the most commonly used drug in CLL. It is used in a daily oral schedule, intermittent schedule, alone, or associated with corticosteroids. Response rates have varied from 27% to 100%, but the interpretation of these data is difficult because of the lack of homogeneity in the groups of patients.\textsuperscript{153-155} The first randomized trial in which patients were selected according to clinical stage failed to observe any significant differences in survival between prednisone (PRD), daily CLB + PRD, and intermittent high dose of CLB + PRD.\textsuperscript{156} In the CLL80 protocol, the FCG on CLL analyzed 612 stage A patients.
randomized between no treatment and daily CLB. Although CLB succeeded in slowing down disease progression and favoring remission, the survival rate in the group treated by CLB was significantly shorter when compared with the other group (P < .05, unpublished updated results). These results were explained by the appearance of an excess of epithelial cancers in the CLB group and the poor prognosis of patients evolving to stages B and C under CLB. After this trial, the FCG tested (in the CLL85 protocol) an intermittent schedule of CLB + PRD versus no treatment in stage A, but results are not yet available. Recently, Jaksic et al[167] have reported the efficiency of high doses of CLB (15 mg/d until complete remission) in advanced CLL. A well-accepted notion regarding CLB is the unfavorable outcome associated with the absence of therapeutic response; this result was used in several trials to segregate benign and active forms of the disease.[5,49-56,58]

Other single agents used in CLL include mainly PRD, busulfan, or cyclophosphamide. Results were often comparable with those observed with CLB alone, though sometimes with more toxicity.[73-75]

Combination Chemotherapy

In advanced forms of the disease, multiple drug regimens have been proposed. The first was COP (cyclophosphamide, vincristine, and prednisone),[18] which gave a response rate of about 70% but failed to show any benefit over CLB alone or CLB + PRD in three randomized trials.[199-201] Other combinations, including MOPP,[202] cytosine arabinoside + cyclophosphamide,[203] M2,[204] CAP,[205] POACH,[206] CMBOX,[207] and M11,[208] have given results generally identical to those obtained with CLB.

In the CLL80 trial, the FCG on CLL randomized 70 stage C patients between COP and CHOP (COP plus doxorubicin 25 mg/m² intravenously [IV] day 1).[189] Median survival was 22 months with COP and 62 months with CHOP, supporting a beneficial effect of low-dose doxorubicin for stage C patients.[189,190] However, Jaksic et al recently found a comparable overall response in “advanced CLL” with CHOP and high-dose CLB,[191] and the Eastern Cooperative Oncology Group has reported a median survival of 49 months in stage C patients treated by CLB + PRD or CVP.[192,193] The CHOP regimen has been recently compared with CLB + PRD in stage B and C patients in randomized trials by the Danish group and the FCG on CLL. The first interim analysis of these protocols showed higher response rates to CHOP than to CLB + PRD,[171,173] but no differences in terms of survival have as yet been observed.

Radiotherapy

Radiotherapy, administered either as P32,[174] total body irradiation,[175] extracorporeal irradiation of blood,[186] or thymic irradiation,[177] was found to be effective in a few patients, but severe myelosuppression is a frequent sequel to this treatment. Splenic irradiation has been more often used, mainly in patients with massive splenomegaly when splenectomy was difficult and the patient had associated autoimmune cytopenias. Recently, the UK Medical Research Council observed a better survival for patients treated by splenic irradiation as compared with CLB or CLB + PRD in two randomized trials, but this difference was not significant.[25,27] Finally, irradiation of large lymph nodes may be used in patients resistant to chemotherapy.

Splenectomy

The main indications for splenectomy are autoimmune hemolytic anemia or thrombocytopenia and massive painful splenomegaly. Although several groups have reported that splenectomy may be an efficient treatment in other groups of patients,[199-201] this has not been shown in randomized studies.

Biologic Response Modifiers

CLL response to antibodies, mainly with CD5 specificity, alone or associated to ricin or an isotope, was recently reviewed, showing only transient efficacy in some patients.[182,184] Although it is effective in vitro[185] in CLL, IFNα has showed a low response rate in vivo except in early stages of the disease.[186,187] Its use once response has been achieved

<table>
<thead>
<tr>
<th>Response</th>
<th>IWCLL Criteria</th>
<th>NCI Criteria</th>
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<tbody>
<tr>
<td>CR</td>
<td>No evidence of disease</td>
<td>Absence of lymphadenopathy, hepatomegaly, splenomegaly or constitutional symptoms. Normal blood count: neutrophils &gt; 1.5 x 10^9/L, platelets &gt; 100 x 10^9/L, Hb &gt; 11 g/dL, lymphocytes &lt; 4.0 x 10^9/L</td>
</tr>
<tr>
<td>PR</td>
<td>Change from stage C to stage A or B; or from stage B to A</td>
<td>50% reduction in blood lymphocytes and 50% reduction in lymphadenopathy and/or 50% reduction in splenomegaly and/or hepatomegaly. Neutrophils &gt; 1.5 x 10^9/L or 50% improvement over baseline; platelets &gt; 100 x 10^9/L or 50% improvement over baseline; Hb &gt; 11.0 g/dL (not supported by transfusion) or 50% improvement over baseline.</td>
</tr>
<tr>
<td>SD</td>
<td>No change in the stage of the disease</td>
<td>No CR, PR, or PD.</td>
</tr>
<tr>
<td>PD</td>
<td>Change from stage A disease to stage B or C, or from stage B to C</td>
<td>At least one of the following: &gt; 50% increase in the size of at least two lymph nodes or new palpable lymphnodes; &gt; 50% increase in splenomegaly or hepatomegaly or appearance if there were not present; transformation to a more aggressive histology Richter or prolymphocytic leukemia; &gt; 50% increase in the absolute number of circulating lymphocytes.</td>
</tr>
</tbody>
</table>
with chemotherapy needs to be investigated. Moderate responses to IL-2 were observed in 7 of 13 patients resistant to chemotherapy; after IL-2, two of those patients receiving chemotherapy had a dramatic response. IL-4, in vitro, has an antiproliferative effect on lymphocytes from B-CLL patients; its efficacy is being currently tested in vivo. Lastly, Tura et al reported prompt remission with cyclosporin A in three cases of CLL-associated pure RBC aplasia.

**New Drugs**

Fludarabine monophosphate is a fluorinated analogue of adenine that is resistant to deamination by adenosine deaminase. Grever et al were the first to report the efficacy of this drug in CLL. Keating et al administered fludarabine 25 or 30 mg/m²/d for 5 days at four weekly intervals to 68 previously treated CLL patients; the response rate was 57% (29% CR and 28% PR). Toxicity was mild and median survival significantly differed between responders and nonresponders. Of 36 previously untreated patients, 75% reached CR. No significantly different results were obtained with fludarabine alone and its combination with corticosteroids. A randomized trial comparing fludarabine with CHOP has recently begun in Europe for stage B and C patients.

A total of 110 patients with advanced diseases and often resistant to chemotherapy were treated by pentostatin (2-deoxycoformycin). The response rate was 25%. This drug is effective but induces a prolonged decrease in T cells, with a possible increase in the risk of infections. Chlorodeoxyadenosine has recently been used in refractory CLL patients; its efficacy is being currently tested in vivo. Grever et al were the first to report the efficacy of this drug in CLL. The period of T-lymphocyte depression after treatment may be shorter than that induced by pentostatin.

**Allogeneic BM Transplantation (BMT)**

A recent study from the European Group for BMT reported 20 patients with advanced disease who received allogeneic BMT (M. Michallet, personal communication, June 1991). Their mean age was 40 years and the conditioning regimen always included total body irradiation. One early death and one early graft failure were observed. The remaining 18 patients all developed acute graft-versus-host disease (GVHD). All entered CR. Seven of them died, three from GVHD, one from hemorrhage, one from late graft failure, and two from relapse (at 7 and 54 months, respectively). Ten patients were alive in CR with a mean of follow-up of 30 months.

**Intravenous Igs**

The fact that hypogammaglobulinemia was the major cause of the increased risk of sepsis in CLL prompted the use of intravenous Igs to prevent infection. In a double-blind randomized study, intravenous Igs (400 mg/kg body weight administered every 3 weeks) reduced the incidence of bacterial infections by 50%. However, the number of severe bacterial infections and viral infections was unaffected and survival was not modified. In addition, because intravenous Igs are an expensive treatment, their role in preventing infection in routine practice in CLL will have to be better defined.

**FUTURE DIRECTIONS**

The first question facing epidemiologists and hematologists is why there is such a low incidence of CLL among Asians. No simple explanation in terms of HLA and other markers is presently available.

Immunologic markers have identified the proliferating lymphocyte in CLL as a mature B lymphocyte that expresses the CD5 marker. It has been postulated that Ly1 B cells (the murine counterpart of human CD5+ B cells) constitute a separate B-cell lineage. Whether the CD5 marker defines a discrete lineage or is a maturation marker is one of the main issues that may be solved in the near future. Another recent advance was the discovery that the CLL B lymphocyte is in an activated state and can be induced to differentiate. Using B-cell mitogens and somatic hybridization, it was shown that the CLL B lymphocyte is frequently involved in the production of natural autoantibodies and expresses a restricted set of genes. These results may provide a basis for passive immunotherapy with antidiotopic antibodies. Several recurrent clonal abnormalities, some associated with prognosis, have been found in about 50% of CLL patients, but none of them is specific for CLL, and no consistent abnormalities at the molecular biologic level have been reported.

Although Rai’s and Binet’s clinical systems succeeded in predicting the evolution in most patients, about 20% of low-risk patients will progress to more advanced disease, and those staging systems are presently unable to predict these evolutions. Predicting progression of low-risk patients will be especially important, because more and more CLL are diagnosed at an early stage. Recent findings suggest that BM histology, lymphocyte doubling time, karyotypic studies, and other biologic parameters will help better define patients who will subsequently progress.

In stage A, it appears reasonable to defer any therapy until disease progression is observed. By contrast, there is general agreement that stages B and C should be treated. Intermittent schedules including chlorambucil and prednisone and intermittent CHOP chemotherapy are the most frequently used regimens. New drugs, especially fludarabin, are promising. The association of these drugs with chlorambucil, doxorubicin, and prednisone is also under study. The role, if any, of biologic response modifiers such as IFNs, IL-2, and MoAbs still has to be defined. Allogeneic BMT gives promising results, but will remain a therapy of exception.

As increasing numbers of complete remissions have now been reported in CLL treated with intensive chemotherapy, autologous BMT using BM harvested in CR is beginning to be investigated in young patients with advanced disease. However, the reality of CR achievement in CLL will have to be substantiated, especially with immunophenotyping and molecular biology techniques. The benefit of achieving CR in terms of survival will also have to be shown.
APPENDIX

Members of the French Cooperative Group on Chronic Lymphocytic Leukemia: CLL protocol.


Coordinating Center: J.-L. Binet (study chairman), CI. Chastang (study biostatistician), G. Dighiero, P. Fenaux, and Ph. Travade (study secretaries).


Writing Committee: J.-L. Binet, CI. Chastang, S. Chevrét, G. Dighiero, P. Fenaux, Ph. Travade.

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