[Internal Medicine Grand Rounds]

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Chronic Lymphocytic Leukemia

New Insights into an Old Problem

Thomas Froehlich, M.D.

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Thomas Froehlich, M.D., M.S.H.A.
Associate Professor
Division of Hematology Oncology

Dr. Froehlich sees patients at the Harold C. Simmons Cancer Center and is the Medical Director of the Hematology Oncology clinics there. He has a particular interest in patients with CLL and malignant lymphomas, especially low grade non-Hodgkin's lymphoma and Hodgkin's disease. He also sees patients with breast cancer, brain tumors and benign hematologic conditions.

Epidemiology

Chronic Lymphocytic Leukemia (CLL) is the most common form of leukemia in adults in Europe and North America. The median age at diagnosis is 72 years but 30% of patients are younger than 64. [1] According to information on the National Cancer Institute's website, it is estimated that there were 14,990 new cases in the US in 2010 and 4390 deaths. CLL is more common in men than women (1.7:1) and the incidence rises significantly as people age.

There are no definite risk factors for CLL that have been identified. While there is no evidence of an inherited cause, approximately 10% of patients with CLL have a first degree relative who has also been diagnosed with CLL or other lymphoproliferative disorder. In addition the only environmental cause that has been linked to CLL is exposure to Agent Orange, which the Veteran's Administration now recognizes as a causative factor in Vietnam Veterans.[2]

Diagnosis

CLL is a malignancy of B lymphocytes with an unusual constellation of all surface markers. CLL cells co-express the T cell antigen CD5 and B cell surface antigens CD19, CD20 and CD23.[3] Levels of surface immunoglobulin and CD20 are low compared to normal B lymphocytes and other B cell malignancies and CLL cells show restriction of surface immunoglobulin to either kappa or lambda light chains. A diagnosis of CLL can be made in patients who have a monoclonal population of B cells with the appropriate constellation of cell surface markers in excess of 5000/ML ($5x10^6$/l) in their peripheral blood.[3] The

lymphocytes are characteristically small, mature appearing lymphocytes with a dense nucleus and minimal cytoplasm. (fig 1) So called "smudge cells" are commonly seen as well. Some patients may present with enlarged lymph nodes infiltrated with cells showing the characteristic surface markers of CLL but without an increase in lymphocytes in the peripheral blood. In these patients, the disease is referred to as small lymphocytic lymphoma (SLL) but the prognosis is similar and most patients with SLL will have abnormal cells in their peripheral blood and ultimately have evidence of leukemia.

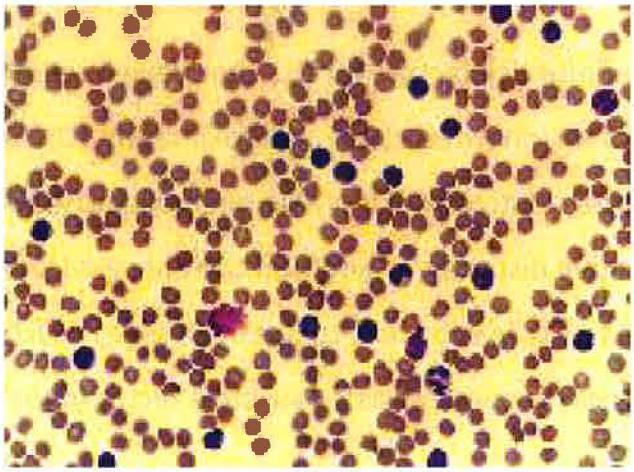

Figure 1-peripheral blood smear in a patient with CLL

The use of flow cytometry to identify monoclonal cell populations in peripheral blood has led to the discovery of individuals who have a population of monoclonal B lymphocytes with the pattern of markers of CLL but whose absolute lymphocyte count is less than 5000/µl. This is now called monoclonal B lymphocytosis (MBL) and patients with this finding progress to frank CLL at a rate of 1-2% per year[4]. However, studies have demonstrated a significantly better survival of patients with MBL than those with early stage CLL.[5]

Staging

Staging of patients with CLL can be done in one of 2 systems, either the Rai or Binet staging [6, 7]. Both systems rely solely on physical examination and routine laboratory tests and both have value in grouping patients by prognosis. The Rai staging system classifies patients who only have lymphocytosis as low risk; those who in addition have lymphadenopathy, splenomegaly or hepatomegaly as intermediate risk and those with anemia (<11gm/DL) or thrombocytopenia (<100,000/µl) as high risk. In the Binet system, blood counts and number of geographic areas of involvement are combined to define stages A, B and C. The area of involvement considered for staging are: 1) head and neck; 2) axillae; 3) groins; 4) palpable spleen; 5) palpable liver that is clinically enlarged. Stage A is defined as Hgb ≥10g/DL, platelets ≥100,000/µl and up to 2 areas of involvement. Stage B is defined by the same hematologic parameters but involvement of 3 or more geographic sites. Stage C is defined by cytopenias, with Hgb <10g/DL or platelets <100,000/µl regardless of the geographic sites of involvement. The groupings of these staging systems have been correlated with significant differences in prognosis, but the variability of survival within the groups is still large and better prognostic indexes are still being sought.[8]

Prognostic Markers

The variation in behavior of CLL among patients has intrigued investigators for years. Most patients' disease behaves as described by Dameshek in 1967 as "an accumulative disease of immunologically incompetent lymphocytes."[9] In fact, many patients diagnosed with CLL will never need treatment for the

disease during their lifetime. However a significant number of patients will have more aggressive disease and progress to treatment more quickly. In addition, some patients have an excellent and prolonged response to initial therapy with regimens such as FCR (fludarabine, cyclophosphamide, rituximab) while others respond poorly or have a brief duration or response to the same treatment. Investigators have found a number of cytogenetic aberrations and protein expression markers that can predict prognosis and response to therapy, but until recently the biology underlying these markers and the behavior of the leukemic cells has been much less clear.

Up to 80% of CLL can be shown to have cytogenetic aberrations using modern techniques.[10] Although conventional cytogenetics are normal in 50-70% of patients, more sensitive analysis using florescence in situ hybridization (FISH) analysis reveals abnormalities in the majority of patients. The most common abnormality is a deletion on the long arm of chromosome 13 (del 13q) and it is a favorable prognostic finding. Less favorable abnormalities include trisomy 12, deletion 11q, deletion 17p and deletion 6q. The finding of del 17p, in particular, is associated with a poor prognosis including poor response to purine analog therapy and short survival.[11-13]

During normal B cell maturation, the genes encoding for the variable regions of immunoglobulin heavy chains (IgVh) undergo mutation, presumably as part of the process of producing antibodies against specific targets. In CLL, mutation of the IgVh locus is strongly associated with a better prognosis while lack of mutation at these loci is associated with a poorer prognosis.[14, 15] In

clinical practice, however, this type of analysis is not routinely available. Because of this, surrogate markers for IgVh mutational status have been sought.

ZAP 70 (zeta associated protein of 70 kilodaltons) expression has been shown to correlate with IgVh status.[16] Untreated patients with unmutated IgVh status typically have higher expression of ZAP 70 seen in microarray analysis and high ZAP 70 expression is associated with a poorer prognosis.[17] Commercial tests for ZAP 70 are available, but it is a labile cell surface molecule, causing reproducibility and reliability of results to be problematic. How blood is handled after it is drawn can significantly affect results, so many clinicians and researchers do not use it in the routine evaluation of patients.

CD38 is a cell surface molecule that is present at various steps of B cell differentiation. The expression of CD38 in CLL cells has been shown to correlate inversely to the presence of mutation of the IgVh genes.[16] Higher levels of CD38 are seen in CLL cells with unmutated IgVh genes and that is associated with a poorer prognosis.[15, 16] Clinical application of this marker has achieved wider use since it is not labile and assessment by flow cytometry is more reproducible.

Biology

The behavior of CLL as a disease of accumulation is now understood as a failure of the normal process of apoptosis. However the cause of this decrease in the natural process of cell death in CLL and its relationship to the described

cytogenetic abnormalities has eluded investigators until recently. But there is a growing body of evidence linking the described abnormalities to underlying biologic processes in CLL cells.

The relationship between CLL cells, bone marrow stromal cells and nurse like cells is important to the survival of CLL cells. In vitro, CLL cells undergo rapid apoptosis but this process can be slowed by the addition stromal and nurse like cells as well as some cytokines such as interferon gamma, interferon alpha and interleukin 13.[18] Both stromal and nurse like cells have been shown to promote CLL survived through signal pathways mediated by APRIL (a proliferation inducing ligand) and BAFF (B-cell activated tumor necrosis factor family) and MMP-9 (matrix metalloproteinase 9).[19, 20]

Genetic Factors

The most common cytogenetic abnormality in CLL, del 13q, involves the loss of a region of DNA that does not encode any genes.[21] However this region does encode for 2 recently detected micro RNA's (miRNA). MiRNAs are 19-25 nucleotide sequences that act to silence target genes via an RNA interference pathway. These 2 miRNAs, known as miR 15A and miR 16-1 are lost in 66% of CLL patients.[22] One target of these miRNAs is the T-cell leukemia gene (TCL-1). The loss of these miRNAs leads to an increase in TCL-1 protein production and its role is to promote cell survival.[22, 23] Deletion of these 2 miRNAs has also been shown to cause an increase in Bcl2, an inhibitor of apoptosis and a strong pro-survival factor.[22]

Another common cytogenetic abnormality, del 17p, directly affects the p53 tumor suppressor gene by causing loss of p53.[24] Since p53 is a key mediator of apoptosis in the presence of DNA damage, deletion or mutation of p53 causes interference with this process which results in decreased apoptosis.[21] Another deletion seen in CLL, del 11q22, leads to a decrease or loss of the ataxia telangiectasia mutated kinase (ATM), whose role is to activate p53, and this also reduces apoptosis.[25] A third mechanism affecting p53 in CLL occurs in trisomy 12. This leads to amplification of the murine double minute protein-2 (MDM2) gene and since MDM2 is a negative regulator of p53 [24], this also leads to reduced apoptosis.

Protein Factors

As already mentioned, ZAP 70 expression is increased in some patients with CLL and is associated with a worse prognosis. ZAP 70 is a receptor associated protein kinase that enhances B cell receptor signaling and promotes cell survival.[26]

Increases in TCL-1 occur in 90% of CLL.[21] The mechanism by which TCL-1 reduces apoptosis is linked to activation of the Akt kinase. Akt phosphorylates the pro-apoptotic protein BAD, which inactivates it, and reduces the rate of apoptosis. Akt also activates NFkB, actually a family of transcription factors, which have anti-apoptotic activity. NFkB also increases Bcl2 protein levels [27] and Bcl2 has been shown to be a survival and proliferation factor for B cells.

Protein kinase R (PKR) is a signal transducer activated in the pro-inflammatory response to extra-cellular stress. It leads to apoptosis.[28] But in 21 of 28 patients with CLL it was found to be nonfunctional.[29] Invitro, inhibition of PKR promotes survival of cells and thus non-functional PKR may be important in the pathogenesis of CLL.[29]

Another kinase, Pi3k (Phosphotidylinositol-3-kinase), is a pro-survival protein. In CLL it's phosphorylation is increased, resulting in activation of this survival pathway.[30] Pi3k activation also increases Akt phosphorylation,[31] which as already discussed, acts to reduce apoptosis by its interactions. In addition to promoting survival, expression of Akt has also been shown to increase proliferation of CLL cells.[30]

MMP9, already discussed in relation to stromal and nurse like cells, is also secreted by CLL cells in increased amounts. In addition to promoting survival, increases in MMP9 have also been shown to facilitate B cell migration and invasion into other tissues.[32] Finally, another pro-apoptosis protein, death-associated protein kinase 1 (DAPK1), has recently been found to be reduced in most B cells derived from CLL patients.[33] This represents another alteration that can potentially reduce normal apoptosis in CLL.

Treatment

For many years oral alkylating agent therapy, most commonly using chlorambucil, was the primary treatment for patients with CLL. Then, in the 1990's, the purine analog fludarabine was shown to be active in CLL and

superior to chlorambucil in patients with CLL.[34, 35] The next advancement of therapy was the combination of fludarabine and cyclophosphamide, shown by the German CLL study group and others to be effective and superior to fludarabine alone.[26, 27]

In 2005, the addition of rituximab to fludarabine and cyclophosphamide (FCR) was shown to be even more effective in untreated patients.[38] However most patients enrolled in trials of this combination have been fit and less than 70 years of age and there are significant toxicities associated with the use of FCR. Since many patients needing treatment are older and have multiple comorbidities, treatment with FCR is not always possible in patients with clear indications for therapy. The combination of pentostatin, cyclophosphamide and rituximab (PCR) has also been shown to effective and may be less toxic [39] but it has not achieved widespread use in the U.S.

Alemtuzumab, a monoclonal antibody against CD52, also displays significant activity in CLL,[40] including relapsed and refractory CLL.[41] However it causes profound immune suppression and infectious complications are common after treatment with alemtuzumab.

New Agents

Bendamustine, an agent that has functional characteristics of both alkylating agents and purine analogs, [42] was approved by the FDA in 2008 based on its efficacy in a trial that compared it to chlorambucil.[43] It has been shown to have activity in patients refractory to chlorambucil and those refractory to

fludarabine [44] so there appears to be little cross resistance between bendamustine and other commonly used drugs in CLL. It is now being used both as a single agent and in combination with rituximab.

Ofatumumab is another anti-CD20 monoclonal antibody, which is directed against a different epitope on the CD 20 molecule than rituximab. It was approved by the FDA in 2010 for the treatment of fludarabine refractory CLL.[45] While it does have activity in this setting as a single agent, duration of response has been short in most patients and it is nearly 10 times as costly as rituximab. Given this fact, how it will ultimately be used in CLL patients is still uncertain.

Another agent, already in use for myeloma, now being explored in CLL is lenalidomide. It is being studied in relapsed and refractory CLL[46] and as consolidation after induction with PCR in previously untreated patients.[47] While results in both settings are promising, lenalidomide has not been approved for either of these indications and because of its cost, unless it is approved for these uses and covered by insurance, it will not achieve wide spread use in CLL.

Two other new agents in early development also look promising. However, the only published data for both of them is phase 1 data. The first is an inhibitor of Pi3k presently known as CAL-101. In preclinical data it appears to have cytotoxic activity in CLL.[48] The second agent, known as PCI-32765, is a highly specific covalent inhibitor of Btk (Bruton's tyrosine kinase), a key enzyme in

the B cell receptor activation pathway. In both phase I trials, these agents were well tolerated and significant activity was seen [48, 49] but development is still in the early stages for both and their ultimate role in treatment of CLL is still to be defined. What is clear is that our new understanding of the underlying cellular mechanisms in CLL is having a strong influence on the development of new agents and new treatment strategies in this common disease. What is less clear at this time is whether this will ultimately lead to a potentially curative strategy for patients with CLL.

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