CHRONIC LYMPHOCYTIC LEUKEMIA

Biology and prognostic factors in CLL

DAVID OSCIER

Department of Haematology, Royal Bournemouth Hospital, Bournemouth, UK

Keywords: Immunoglobulin variable region genes, B cell receptor, ZAP 70

The biology of CLL

The traditional view of CLL as a slowly accumulative disease of immuno-incompetent lymphocytes is being superseded as new data reveals biological heterogeneity both between patients and in individual cases at different times in the course of their disease. Some of the recent key advances are summarized below:

The B cell receptor

The immunoglobulin heavy and light chain genes which encode the antigen binding site of the B cell receptor (BCR) in CLL may be either mutated or unmutated raising the question as to whether CLL originates from a single, or more than one target cell. Microarray studies performed to address this issue have been inconclusive but do show a common “CLL signature” quite similar to that of both normal naïve unmutated B cells and mutated memory B cells. However, the pattern of surface antigen expression which is consistent with an activated B cell, the presence of short telomeres reflecting multiple cell divisions, and the finding of biased IgVH gene usage all suggest that unmutated CLL cells have been exposed to antigen. The analysis of IgVH gene usage in large numbers of patients with CLL has recently revealed that there are several sub-groups of patients (comprising approximately 10% of all cases of CLL) that have highly or moderately homologous CDR3 regions, encoded by V D and J genes, and critical for antigen binding. There is a <1 in 10^6 chance of normal B cells having identical CDR3’s, suggesting a role for specific antigen(s) in the pathogenesis of CLL. Whether antigen is responsible for selecting B cell clones for expansion and increasing the chance of a transforming event or is responsible for the subsequent survival and expansion of the leukaemic clone remains uncertain.

The ability of CLL cells to signal through the BCR is diminished as compared to normal B cells, and is especially poor in the mutated subgroup. There are a number of hypotheses to account for this difference in signalling between the two mutational subsets. These include an inability to bind antigen through the BCR, and/or a defect in the organization of the BCR in mutated cases, and the expression of Zap 70, a key molecule in T-cell signalling, in the majority of unmutated cases. The increased cell turnover in unmutated CLL may favor the acquisition of poor prognostic secondary cytogenetic abnormalities.

Genetic abnormalities

Cytogenetic analysis, interphase FISH and comparative genomic hybridization have identified genetic abnormalities in approximately 80% of patients with CLL. The incidence of abnormalities is higher in patients with advanced disease. The nature of the initial transforming events remains unknown. Transgenic mice which over-express the TCL1 gene develop a CD5+ B cell lymphocytosis reminiscent of CLL, but there is no evidence that TCL1 over-expression is an initiating event in human CLL. In contrast to B-cell tumours arising from germinal centres, translocations involving the immunoglobulin gene loci are rare in CLL, occurring in approximately 5% of patients. The commonest genetic abnormality in CLL is deletion of chromosome 13q14. Heterozygous or homozygous loss is found in up to 70% and 20% of cases respectively depending on the methodology used for their detection. Of particular interest is the finding of 13q14 loss as the sole cytogenetic abnormality in cases of ‘preclinical’ CLL described below, confirming that this is an early event in
leukaemogenesis. A minimally deleted region containing exons of two genes, RFP2 and DLEU 2, and two micro RNA’s, miRNA 15 and 16, has been described but their role of 13q loss in the pathogenesis of CLL is not clearly established. Trisomy of chromosome 12, deletion of chromosome 11q23 resulting in loss of the ATM gene and structural abnormalities of chromosome 17p13 resulting in loss of the p53 gene are recurring abnormalities whose incidence varies depending on the clinical stage and IgVH gene mutation status of the disease.

Approximately 5% of patients with CLL have a first degree relative with a chronic lymphoid malignancy and research is ongoing to identify "familial" CLL gene(s) which may also be important in sporadic CLL.

**Role of the micro environment**

The apparent longevity of CLL cells in vivo is in marked contrast to the rapid apoptosis which occurs in vitro. This paradox is accounted for by the discovery of an increasingly complex series of interactions between receptors on the surface of CLL cells and a variety of cell types such as activated T-cells, mesenchymal stromal cells and follicular dendritic cells. Histological and immunophenotypic studies of lymph nodes and bone marrow in CLL has revealed the presence of proliferation centres or pseudofollicles. The immunophenotype of leukaemic cells within these centres differs from that of circulating CLL cells in the increased expression of the proliferation marker Ki67, the chemokines CCL17 and CCL22, and the anti-apoptotic factor, surviving. Whether unmutated CLL cells are better able to access, or to benefit from the proliferative and anti-apoptotic stimuli provided by this microenvironment is unclear.

**Cellular kinetics of CLL**

CLL is classically described as a slowly accumulative disease of functionally incompetent B-cells consequent upon defective apoptosis. This view has been challenged by data in which patients drank “heavy water” for an 84-day period and in whom the incorporation of 2H into lymphocyte DNA was measured both during and after the labelling period. Surprisingly the leukaemic cell birth rate varied from 0.1% up to 1% of the entire CLL clone per day and higher birth rates correlated with disease progression.

**Pre-clinical CLL**

The development of sensitive flow cytometric assays for detecting minimal residual disease in CLL patients enabled this assay to be applied to normal individuals with no lymphocytosis. Small clonal B cell populations with the same immunophenotype as CLL could be detected in approximately 5% of individuals over the age of 60 and in 12% of normal relatives who had family members with CLL. IgVH gene analysis shows these clones to have mutated VH genes. The reason why only a minority of patients with pre-clinical CLL evolve into a recognizable disease remains to be discovered, but could reflect the acquisition of secondary genetic abnormalities and/or access to a permissive microenvironment.

**Prognostic factors**

The potential value of prognostic factors is 2-fold: firstly to predict the natural history of the disease and secondly to predict the response to therapy. Traditionally therapeutic decisions in CLL have been based on staging systems which largely reflect tumour burden. The majority of patients now present with a low tumour burden characterized by a lymphocytosis with or without non-bulky lymphadenopathy and additional markers are required to predict their clinical course. A list of prognostic factors is shown in the table.

Many studies have documented that the median survival of patients with unmutated IgVH genes is 8–10 years compared to over 20 years for those with mutated IgVH genes. There is still uncertainty whether a 97% or 98% homology to the germline sequence is the better discriminator between the 2 mutational subsets. It is also recognised that VH gene usage as well as mutational status affects clinical outcome. Patients utilising the VH3-21 gene, regardless of mutational status, have a survival comparable to cases of unmutated CLL. Although IgVH gene sequencing is unsuitable for routine laboratories, gene expression profiling in CLL has identified a series of genes which are differentially expressed in the 2 mutational subsets. Of these ZAP70 is the most studied and shows 80–90% concordance with IgVH gene status. There is currently an international collaboration to develop and adopt a standardized flow cytometric assay for ZAP70.

Numerous studies have shown that high expression of CD38 correlates with poor outcome in CLL. Results are discordant with IgVH gene status in approximately 30% of cases and it is uncertain whether CD38 expression has prognostic significance in multivariate analyses which include either IgVH gene mutations or ZAP70 expression.

Cytogenetic and interphase FISH studies have clearly shown the prognostic importance of cytogenetic abnormalities. Cases with a normal karyotype or del 13q14 as the sole karyotypic abnormality have a significantly better survival than cases with del 11q23 or del 17p13. The prognostic significance of trisomy 12 remains unclear. Loss and/or mutation of the p53 gene is found in approximately 5% of patients at presentation but in 30% of patients with fludarabine
refractory disease. Identification of p53 abnormalities by FISH, screening for mutations or a functional assay, in which upregulation of p53 and p21 is assessed following in vitro DNA damage, is of particular importance in CLL since loss or mutation of this gene predicts for poor response to alkylating agents, purine analogues and single agent Rituximab. ATM loss in cases with del 11q23 is accompanied by mutation of the ATM gene on the remaining allele in approximately 30% of cases. However ATM mutations may also be found in patients without cytogenetic or interphase FISH evidence of ATM loss. The comparative prognostic significance of ATM loss and ATM mutation is still being evaluated.

To date, a series of retrospective studies have shown that a panel of prognostic markers can predict both clinical course and for p53, response to therapy. These results are now being validated in prospective randomized trials and should demonstrate whether early intervention in asymptomatic patients with poor risk disease is of clinical benefit. The final challenge will be to identify prognostic factors that are sufficiently robust to apply to the management of individual patients.