the other evolves from more differentiated B cells (memory B-cells) with somatically mutated heavy chain genes and has a good prognosis.[10-12] However, recent data derived from gene expression profiling analysis failed to clearly distinguish unmutated and mutated cases and favor the view that all cases of CLL have a common cell origin and/or a common mechanism of malignant transformation.[13,14] A recent study from our group on 18 cases of CLL, compared 9 Binet stage A cases with stable disease (*i.e.* at least 5 years without treatment and any evolution) and mutated Ig genes to 9 cases with stage B or C aggressive disease and unmutated Ig genes. In agreement with previous reports indicating that Ig mutated and unmutated cases have globally the same gene expression pattern, a supervised statistical analysis showed that only 85 genes were differently expressed by a factor >2 between the two groups of CLL. However, in contrast to previous reports, a non supervised hierarchical clustering analysis could clearly separate the stable mutated group from the aggressive unmutated one, except for one case (*Davi et al., ASH meeting 2002*).

These results show that gene expression profiling can distinguish CLL cases with a stable evolution and mutated Ig genes from those with unmutated Ig genes and progressive disease. Thus, monitoring the expression of a very limited number of genes might suffice to identify patients displaying an indolent disease from patients exhibiting an aggressive one.

Guillaume Dighiero
Institut Pasteur, Paris

Note

This paper was presented at the Congress on "Chronic lymphocytic leukemia: is it a curable disease?", held in Bologna, Italy, on October 10-11, 2002. Meeting proceedings can be downloaded at the following URL: http://www.haematologica.org/free/CLL2002.pdf.

References

1. Schroeder HW Jr, Dighiero G. The pathogenesis of chronic lymphocytic leukemia: analysis of the antibody repertoire. Immunol Today 1994; 15:288-94.
2. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood 1975; 46:219-34.
3. Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, Goasguen J, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 1981; 48:198-206.
4. Dighiero G, Maloum K, Desablens B, Cazin B, Navarro M, Leblay R, et al. Chlorambucil in indolent chronic lymphocytic leukemia. French Cooperative Group on Chronic Lymphocytic Leukemia. N Engl J Med 1998; 338:1506-14.
5. Anonymous. Chemotherapeutic options in chronic lymphocytic leukemia: a meta-analysis of the randomized trials. CLL Trialists' Collaborative Group. J Natl Cancer Inst 1999; 91:861-8.
6. Keating MJ, Lerner S, Kantarjian H, Freireich EJ, O'Brien S. The serum β2-microglobulin (β2M) level is more powerful than stage in predicting response and survival in chronic lymphocytic leukemia (CLL). Blood 1998; 86:606a [abstract #2412].
7. Sarfati M, Bron D, Lagneaux L, Fonteyn C, Frost H, Delespesse G. Elevation of IgE-binding factors in serum of patients with B cell-derived chronic lymphocytic leukemia. Blood 1988; 71:94-8.
8. Hallek M, Langenmayer I, Nerl C, Knauf W, Dietzfelbinger H, Ador D, et al. Elevated serum thymidine kinase levels identify a subgroup at high risk of disease progression in early, nonsmoldering chronic lymphocytic leukemia. Blood 1999; 93:1732-7.
9. Dohner H, Stilgebauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000; 343: 1910-6.
10. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999; 94:1840-7.
11. Hamblin TJ, Davis Z, Oscier DG, Stevenson FK. Unmutated immunoglobulin VH genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999; 94:1848-55.
12. Maloum K, Pritsch O, Magnac C, Vuillier F, Davi F, Troussard X, Mauro FF, et al. Expression of unmutated VH genes is a detrimental prognostic factor in Chronic Lymphocytic Leukemia. Blood 2000; 96:377-9.
13. Klein U, Tu Y, Stolovitzky GA, Mattioli M, Cattoretti G, Husson H, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. J Exp Med 2001; 194: 1625-38.
14. Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 2001; 194:1639-47.

Is chronic lymphocytic leukemia one disease?

The most obvious reason for thinking that chronic lymphocytic leukemia (CLL) comprises two diseases, similar to each other but distinguishable, is the fact that some patients are killed by it and some are not. Furthermore, this heterogeneity is not a gradual blend from one to the other, but a distinct demarcation. Even more convincing is the fact that the allocation to one camp or the other is predictable from the date of diagnosis. The surest discriminator is the presence or absence of somatic mutations in the immunoglobulin variable region (*IgV*) genes. The very fact that most experts used to think of CLL as a tumor of naïve B-cells reinforces this concept.[1] Experts work in tertiary referral centers. Tertiary referral centers see

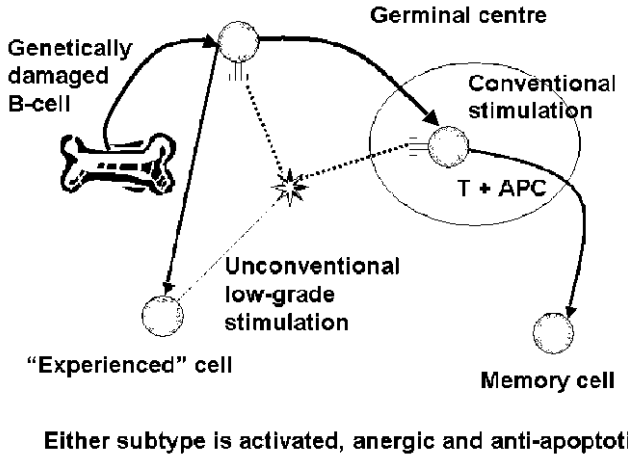

Figure 1. Possible mechanisms of CLL tumorigenesis.

the most serious diseases. CLL is only a serious disease when it has unmutated IgV genes. Schroeder and Dighiero's observation[2] that at least half of CLLs had somatic mutations came as a surprise to the hematologic community only slightly surpassed by the report from two groups that patients whose tumors had somatic mutations had a median survival of 25 years compared to eight years for those whose tumors did not.[3,4] This distinction holds with the accumulation of many more cases in the literature.

Unmutated CLL is mainly a tumor of males, but mutated CLL occurs equally commonly in either sex. The two subtypes differ in their use of *IgV* heavy chain genes. The 51p1 polymorphism of *V1-69* is predominately used by the unmutated subset[1,3,5] while *V4-34* and *V3-23* are almost confined to the mutated subset.[3,5] These are the commonest genes used but similar biases are found with several other genes. A strange anomaly is the use of the *V3-21* gene,[6] especially when used with the JH6 gene. In these cases the CDR3 is very short and even those with somatic mutations have a poor prognosis.

The two subtypes also differ in the expression of CD38,[4] an activation antigen usually present in the unmutated subtype and much less commonly in the mutated subset. Karyotypically, the two subsets differ. The mutated subset either a normal karyotype or deletion at 13q14,[7] whereas the unmutated subset is more likely to have trisomy 12,[7] and deletions at 11q23 or 17p15 are almost confined to the unmutated subset.[8,9] There are also physiologic differences in that signaling through IgM (though not IgD) is deficient in the mutated subset but normal in the unmutated subset.[8]

The obvious explanation for the difference was the hypothesis that those cases with somatic mutations arose from a cell that had encountered antigen in the context of the germinal center, while those without somatic mutations were derived from a cell that had not encountered antigen. There are reasons for doubting that this is a complete explanation. First, a number of laboratories have questioned the demarcation between mutated and unmutated subsets at 98% homology with the germ line sequence.[9-11] Ninety-eight percent was chosen because this degree of variance could be caused by the polymorphisms known to be present in these genes, and because other (unknown) polymorphisms were suspected. If diseases with less homology behave badly then the influence of the germinal center becomes questionable. The truth of this assertion awaits discovery of all the polymorphisms. Investigation of the most appropriate demarcation line demands that deaths unrelated to CLL be discarded from analysis. Patients presenting with advanced disease should also be excluded because an unknown period of asymptomatic disease may have preceded discovery. In our hands the 98% cut-off is still the best.

Second, both subsets express CD27 equally.[12] CD27 is an antigen identified with memory B-cells suggesting that both subsets have been exposed to antigen. There is something of a circular argument here, because CD27 was originally identified as a marker for memory B cells because it was found on cells with mutated IgV genes.[13]

Third, gene expression data from two laboratories[14,15] demonstrate that the two subtypes are more similar to each other than to any other B-cell tumor or to any type of normal B-cell, though they are more akin to memory B-cells than any other type of normal cell tested. However, they are distinguishable from each other by the expression and

non-expression of several genes. One gene that seems to distinguish the two subtypes is Zap-70, which codes for a signal transduction molecule used by T-cells and inexplicably upregulated in unmutated CLL. Protein expression can be measured by flow cytometry and high levels confer a poor prognosis.[16]

It envisages an intrinsic defect in all CLLs whereby stimulation of the B-cell receptor (BCR) induces a reaction pattern of partial activation, anergy and failure of apoptosis.[17] In the mutated subgroup stimulation of the BCR takes place conventionally within the germinal center. The cell would be destined for apoptosis, were that possible, but instead remains a slowly accumulating, indolent tumor. In the unmutated subgroup stimulation of the BCR takes place outside the germinal center, whether by a T-independent antigen or superantigen. A similar succession of events occurs, but re-stimulation is likely leading to a slow succession of cell divisions. Every extra cell division exposes the cell to further genetic damage. The acquisition of abnormalities such as mutations of ATM[18] or p53[8-10] releases the cell from proliferative constraints, leading to a more malignant process.

Although the two subsets have identifiably different characteristics — sex ratio, karyotype, biased use of IgVH genes, ability to signal through surface IgM, CD38 expression — none of these characteristics corresponds precisely with somatic mutation status. Having or not having somatic mutations decreases or increases the risk of further deleterious consequences. Perhaps the risk is related to the rate of cell division.

Finally, the most telling factor is the predictability by IgVH gene analysis at diagnosis that a patient will fall into the benign or malignant subgroup. We have looked at the stage A' described by the French Cooperative Group. These patients were predicted to have a survival curve similar to that of the general population. It is known, however, that about 25% will progress at 5 years. In our hands IgVH gene sequencing provides the most reliable indicator that stage A' cases will progress so as to require treatment.

An alternative explanation for the absence of somatic mutations in some CLLs is the presence of a crippled mutator enzyme system. Several groups have been examining activation-induced cytidine deaminase (AID) in CLL.[19-21] This enzyme is responsible for the initiation of both class switching and somatic hypermutation of the immunoglobulin molecule.[22] A common finding is a paradoxical upregulation of AID mRNA, mainly in the unmutated subset. It is likely that this is a reaction to a blocked pathway. One group suggests that splice variants of AID form inactivating heterodimers with the wild-type enzyme.[19]

Implications

Current practice is to leave Binet stage A or Rai stage 0 CLL untreated until progression occurs. A meta-analysis of clinical trials shows no benefit for up-front chlorambucil treatment versus delayed therapy in these groups of patients.[22] We now have more effective treatments than chlorambucil that are capable of obtaining molecular complete remissions even in advanced disease. We should consider new clinical trials comparing, among the unmutated subgroup, treatment with curative intent with conventional watch-and-wait protocols.

Terry Hamblin
Royal Bournemouth Hospital,
Bournemouth, BH7 7DW, UK

Acknowledgments

This work is the product of co-operation with Freda Stevenson, David Oscier, Jenny Orchard, Rachel Ibbotson, Zadie Davis, Anne Gardiner and Stuart Lanham. Funding was provided by Tenovus, Leukaemia Research Fund and the Bournemouth Leukaemia Fund.

Note

This paper was presented at the Congress on "Chronic lymphocytic leukemia: is it a curable disease?", held in Bologna, Italy, on October 10-11, 2002. Meeting proceedings can be downloaded at the following URL: http://www.haematologica.org/free/CLL2002.pdf.

References

1. Kipps TJ, Tomhave E, Pratt LF, Duffey S, Chen PP, Carson DA. Developmentally restricted immunoglobulin heavy chain variable region gene expressed at high frequency in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 1989; 86:5913.
2. Schroeder HW Jr, Dighiero G. The pathogenesis of chronic lymphocytic leukemia: analysis of the antibody repertoire. Immunol Today 1994; 15:288-94.
3. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999; 94:1848-54.
4. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999; 94:1840-7.
5. Fais F, Ghiotto F, Hashimoto S, Sellers B, Valetto A, Schulman P, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest 1998; 102:1515.
6. Tobin G, Thunberg U, Johnson A, Thorn I, Soderberg O, Hultdin M, et al. Somatically mutated Ig V(H)3-21 genes

characterize a new subset of chronic lymphocytic leukemia. Blood 2002; 99:2262-4.
7. Oscier DG, Thompsett A, Zhu D, Stevenson FK. Differential rates of somatic hypermutation in VH genes among subsets of chronic lymphocytic leukemia defined by chromosomal abnormalities. Blood 1997; 89:4153-60.
8. Oscier DG, Gardiner AC, Mould SJ, Glide S, Davies ZA, Ibbotson RE, et al. Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors Blood 2002; 100:1177-84.
9. Krober A, Seller T, Benner A, Bullinger L, Bruckle E, Lichter P, et al. VH mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. Blood 2002; 100:1410-6.
10. Lin K, Sherrington P, Dennis M, Matrai Z, Cawley JC, Pettitt AR. Relationship between p53 dysfunction, CD38 expression, and IgVH mutation in chronic lymphocytic leukemia. Blood 2002; 100:1404-9.
11. Nollet F, Cauwelier B, Billiet J, Sellesiag D, Van Hoof A, Louwagie A, et al. Do B-cell chronic lymphocytic leukemia patients with IgVH 3-21 genes constitute a new subset of chronic lymphocytic leukemia? Blood 2002; 100:1097-8.
12. Damle RN, Ghiotto F, Valetto A, Albesiano E, Fais F, Yan XJ, et al. B-cell chronic lymphocytic leukemia cells express a surface membrane phenotype of activated, antigen-experienced B lymphocytes. Blood 2002; 99:4087-93.
13. Klein U, Rajewsky K, Kuppers R. Human immunoglobulin (Ig)M^+ IgD^+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. J Exp Med 1998; 188:1679-89.
14. Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 2001; 194:1639-47.
15. Klein U, Tu Y, Stolovitzky GA, Mattioli M, Cattoretti G, Husson H, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. J Exp Med 2001; 194: 1625-38.
16. Orchard JA, Ibbotson RE, Davis ZA, Gardiner AC, Hamblin TJ, Oscier DG. Zap-70 evaluation by flow cytometry is a significant prognostic marker in B-CLL. Blood 2002; 100 Suppl 1:[abstract](in press).
17. Caligaris-Cappio F, Hamblin TJ. B-cell chronic lymphocytic leukemia: a bird of a different feather. J Clin Oncol 1999 17:399-408.
18. Stankovic T, Stewart GS, Fegan C, Biggs P, Last J, Byrd PJ, et al. Ataxia telangiectasia mutated-deficient B-cell chronic lymphocytic leukemia occurs in pregerminal center cells and results in defective damage response and unrepaired chromosome damage. Blood 2002; 99:300-9.
19. McCarthy H, Wierda WG, Barron LL, Cromwell C, Keating MJ, Abruzzo LV. CLL patients with unmutated immunoglobulin VH genes express high levels of activation-induced cytidine deaminase (AID) mRNA and splice variants. Blood 2002; 100 Suppl 1:[abstract](in press).
20. Albesiano E, Messmer BT, Damle RN, Allen SL, Kudalkar PR, Koilitz JE, et al. Activation-induced cytidine deaminase (AID) expression in B-CLL. Blood 2002; 100 Suppl 1: [abstract](in press).
21. Oppezzo P, Vuillier F, Vasconcelos Y, Magna C, Dumas G, Payelle-Brogard B, et al. CLL-B-cells expressing AID display a dissociation between class switch recombination and somatic hypermutation. Blood 2002; 100 Suppl 1: [abstract](in press).
22. Anonymous. Chemotherapeutic options in chronic lymphocytic leukemia: a meta-analysis of the randomized trials. CLL Trialists' Collaborative Group. J Natl Cancer Inst 1999; 91:861-8.

Management of primary mediastinal B-cell lymphoma with sclerosis: advances and caveats

Primary mediastinal B-cell lymphoma with sclerosis is a distinctive subtype of non-Hodgkin's lymphoma with unique clinicopathologic aspects and aggressive behavior.[1] In 1997 Lazzarino *et al*.[2] reported studies on 106 patients, 99 of whom received doxorubicin-containing chemotherapy. Thirty-five of 99 patients were primarily chemotherapy-resistant, and 64 responded: most of the responders received mediastinal radiotherapy. The actuarial 3-year survival rate was 52% for all patients and 82% for responders. Poor performance status and pericardial effusion predicted non response and poor survival. Inadequate response after the first courses of front-line chemotherapy predicted failure of subsequent treatment. Responders with a bulky mediastinum or residual mediastinal abnormality after chemotherapy were at risk of relapse. Retrospective studies are useful in trying to define the natural history of rare disorders, and this may apply to primary mediastinal B-cell lymphoma with sclerosis, which represents about 3% of non-Hodgkin's lymphomas. However, retrospective studies have major drawbacks, and one limitation in this case might be the appropriateness of the histologic diagnosis.[3] It cannot be excluded that retrospective studies include cases of diffuse large cell lymphoma that have a more favorable prognosis.

There is no question that primary mediastinal B-cell lymphoma with sclerosis requires aggressive treatment. In a report in this journal on 89 patients, Zinzani *et al*.[4] showed that combined modality treatment using the MACOP-B chemotherapy regimen and radiation therapy induced a good remission rate with the patients having a greater than 90% chance of surviving disease-free at 9 years. They also emphasized that radiotherapy often plays a pivotal role in obtaining complete remission status. In this issue, Zinzani *et al*.[5] report observations on 426 patients diagnosed with primary mediastinal B-cell lymphoma with sclerosis in 20 institutions from different countries. This retrospective study strongly suggests that MACOP-B (or similar third-generation chemotherapy regimens such as VACOP-B) plus radiation therapy represents the best therapeutic option for most of these patients, with the long-term overall survival being as high as 70-75%. On the other hand, patients with predic-