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The continuing search for the cell of origin of chronic lymphocytic leukemia



Introduction

For long time, chronic lymphocytic leukemia (CLL) has been considered to be a clonal accumulation of immature, immune-incompetent B lymphocytes which accumulate in the body mainly because of a faulty apoptotic apparatus. Numerous recent findings have contributed to a change in this classical credo.¹⁻³ CLL is now viewed as a disease of antigen experience B cells, which have proliferated and possibly continue to do so in response to an antigenic stimulus in some instances even following the final transforming events. Several findings, which take place, have contributed to the formulation of this hypothesis, including the impossibility of detecting lesions in the cell apoptotic apparatus,⁴ the studies on telomeres length of the neoplastic cells,⁵ which demonstrated shorter telomeres than in normal antigen-inexperienced B cells, and the cell turn-over studies using deuterium to label the cell nucleic acids.⁶ The latter studies have demonstrated that CLL cells have a rather rapid turn-over characterized by active cell proliferation and death. This turn-over may be different in different patients and in general is more active in those patients whose cells utilize unmu-

tated VDJ gene segments and express CD38 and ZAP-70. Altogether, the latter findings also raise the question of whether CLL is an homogeneous disease entity, or whether it should be considered as being comprised of two different pathological conditions, one (the unmutated CLL) with a rather aggressive clinical course and outcome related to a more active and invasive clonal expansion and the other (mutated CLL) with a more indolent clinical course and the tendency not to progress towards the more advanced clinical stages. Here, we shall review the immunologic features of the neoplastic cells, including some facts and speculations on the possible encounters with antigen. Based on this and other information, we shall try to indicate the potential progenitor cell of the CLL. Central to this question is also the issue of whether one or more cell types can generate CLL clones.

Organization of the mature B cell system

The possibility of investigating the structure of the genes encoding for Ig H and L chains offers opportunity of elucidating the past history of B cells, including neoplastic cells. In a normal immune response, virgin B cells

encounter stimulating antigens in the T cell dependent areas of peripheral lymphoid tissues, and, with the help of T cells, other accessory cells and cytokine, begin to proliferate and move towards the germinal centers (GC) of secondary lymphoid follicles. Here, the activated B cells continue to proliferate under the stimulus of the antigen they have first encountered and begin to accumulate point mutations in the VDJ and VL gene segments encoding for the variable region of the H and L chains of the antibody molecule respectively. Because of this ongoing mutation process in proliferating B cells, a marked cellular heterogeneity will be created. The B cell receptor (BcR) of certain cells will lose the capacity to recognize the stimulating antigen, whereas that of others will continue to bind the antigen with an even higher affinity. This selective process in the GC results in the survival and expansion of B cells producing high affinity antibodies and in the loss of those B cells not capable of producing these antibodies. Most of the times, there is a concomitant isotype switch, whereby a stimulated/selected cell will change from IgM to IgG or IgA synthesis, while maintaining the same antibody specificity. Once exited from the GC, the selected B cells will become antibody secreting plasma cells or memory cells. The latter cells seed most often in the marginal zone (MZ) of the spleen or in the MZ-like areas of the other peripheral lymphoid tissues. Another pathway of mature B cell differentiation involves their stimulation in the MZ of the spleen or in the MZ-like areas of the peripheral lymphoid tissues. In these conditions, the stimulation occurs mainly in a T cell independent manner, particularly by antigens constituted by polysaccharides or having polysaccharide moieties. The cells so stimulated rarely undergo isotype switch and may accumulate, although not necessarily, somatic mutations in their VDJ and VL gene segments.

The previous history of chronic lymphocytic leukemia clones

Based upon the above scheme, it is apparent that the cells from mutated CLL derive from antigen-experience cells, given the prerequisite for antigenic stimulation for an accumulation of V gene somatic mutations to occur. The issue of whether unmutated CLL cells derive from virgin or antigen-experienced B cells was approached by repertoire studies, comparing the repertoire of normal virgin B cells with that of CLL cells. The rationale for this approach is that, if antigen stimulation/selection occurs in the CLL clones possibly prior or in the course of neoplastic transformation, then the CLL repertoire (intended as the VDJ and VL gene usage by a large cohort of CLL clones) should be different from the same repertoire detected in normal virgin B cells. Indeed, studies on more than a thousand patients demonstrated that CLL cells use a skewed repertoire compared to normal. In addition, the peculiarity of this repertoire is represented by the fact that more⁷⁻¹¹ than 20% of the “unmutated” neoplastic clones utilize a restricted set of stereotyped BcR which are encoded for by the same VDJ and VL segments and share the same combining site. Taken together, these findings lead to the inescapable conclusion that different clones with shared BcR were exposed to the selective pressure of the same antigenic stimulation, that has “chosen” certain cellular specificities among myriads of possible combinations. This conclusion also is supported by the consideration that the chance that two neoplastic clones share BcR with the same specificity is less than 1 in 10⁶, given the present knowledge on the molecular mechanisms controlling the gene encoding for H and L chain V region gene repertoire. Most of the receptors of the unmutated CLL clones have “natural antibody” or “polyspecific” reactivity, i.e. an antibody produced by a single CLL clone can react

with several antigens, including auto-antigens. This observation suggests that antigenic stimulation may continue following neoplastic transformation and promotes clonal expansion, a consideration that may be helpful in explaining why patients with unmutated CLL clones have a more aggressive clinical course and outcome than those with mutated clones which usually do not have BcR with polyspecific activity.^{1,2}

Chronic lymphocytic leukemia.

One or two cell type origin?

At first sight, the above considerations suggest that the two major types of CLL (mutated and unmutated) originate from two different cells, with distinct development and histories of antigen stimulation. Support to this notion is provided by the very different Ig VH/VL gene repertoire of the two CLL subsets; in one case, the cells use a mutated set of genes and do not exhibit evidence of shared usage of stereotyped receptor, while in the case of unmutated CLL, shared use of stereotyped receptors is often the rule. In addition, the presence of somatic mutations of VDJ/VL gene segments, that in some instances could be abundant, suggest passage/ selection of B cells through the germinal centers. In contrast, selection of a repertoire of unmutated, stereotyped gene may be indicative of a stimulation operated by a restricted set of T cell independent antigens. This stimulation, often, prevents both accumulation of point mutations and isotype switch.

There are, however, a number of consideration which still support the notion that of a common cellular origin of the two CLL subsets. For example, gene expression analyses reveal substantial similarities between the cells of the two CLL subsets, both having gene signatures sharing many similarities with normal memory B cells and not with normal virgin B cells.¹² In addition, only unmutated CLL express BcR with natural antibody activity;

however, experiments of site directed mutagenesis have demonstrated that abrogation of somatic mutations in VH/VL genes from mutated cases results in their acquisition of the capacity of encoding for antibodies with natural antibody activity.¹³ This represent a strong support to the notion that the ancestor cells of CLL were employing a common set of VH/VL genes (encoding for natural antibodies). Because of subsequent event(s) this initial set of genes diverged along a different pathway in different cellular clones.

From genes to cells: models to explain the cell of chronic lymphocytic leukemia origin

Based upon the above considerations, several models could explain the cellular origin of CLL. These models can be divided into those that invoke a single cell origin and those that propose an origin from at least two cellular subsets.

Single cell origin

As stated above, this idea is supported primarily by the observation of GEP similarities between cells from different CLL subsets and the derivation of the BcR genes from a single set of progenitors. Based on these premises, the model that most likely can explain the CLL origin is that neoplastic cells derive from MZ B cells that were stimulated by antigen in a T cell independent fashion.¹ Given the differences in repertoire and somatic mutations in the Ig V region genes, one has to assume that the pathway leading to neoplastic transformation differ quite early in leukemogenesis. For example, considering the advantage conferred by the utilization of unmutated BcR to the clonal expansion, it is mandatory to believe that VH gene somatic mutations occurred before neoplastic transformation in mutated CLL. These differences can be in part account-

ed for by differences in the nature of the antigenic stimulus causing clonal expansions before neoplastic transformation in the two CLL subsets. In addition, this antigenic stimulation could have exerted its action on two different MZ B cell subsets following two different pathways of subsequent differentiation. Unfortunately, the information we have so far on human MZ B cells as well as on their VH/VL gene repertoire are too scanty to be able to reach firm conclusions on these issues.

Multiple cell origin

The hypothesis implies that CLL is comprised of at least two different disease entities originating from different cells. Therefore, the options we have regarding this hypothesis should be dealt with separately for each of the two CLL subsets.

Unmutated chronic lymphocytic leukemia

The major feature of these aggressive CLL form is the utilization of a restricted set of stereotyped receptors by a substantial proportion of cases. Therefore, the discovery of normal B cells with these features would lead to the identification of the cell of origin of CLL. However, such normal cells subset has not as yet be found in any of the B cell population examined by us and others, including peripheral blood, tonsil, spleen and peritoneal cavity B cells. This may suggest that a “pre-selection” process has occurred during leukemogenesis, whereby special antigens have selectively induced the expansion of a restricted number of clones which subsequently have undergone neoplastic transformation. This hypothesis implies a close connection between antigenic stimulation and leukemogenesis; whether the antigen has only a promoting effect in this process, facilitating clonal expansion, or whether it has also a transforming effect is open to speculation. According to the latter view, and in a rather speculative perspective,

the stimulating antigen could be a viral particle capable of both stimulating B cells and concomitantly infecting them to induce many of the needed transforming events. In the mouse, there is subset of B cells characterized by a restricted repertoire usage, which respond to T cell independent antigens and autoantigens, have a self-renewing capacity, release Ig mainly of the IgM isotype with natural antibody activity, and are detected primarily in the peritoneal cavity. These cells, which are considered to represent a first line of defense against incoming pathogens in hosts that do not possess as yet an immunological memory, are defined as B1 cells to distinguish them from “classical” B cells producing high affinity antibodies mainly of the IgG class.¹⁴ The latter B cells are defined as B2 cells. In man, B1 cells have still to be detected, although it is possible that they expand only under particular environmental conditions, which may be frequent for mice kept in captivity, but infrequent for humans. Accordingly, CLL could be generated by the human equivalent of B1 cells and, for the reasons explained above, the transforming events are likely to be preceded by other event(s) facilitating the expansion of these cells. It is also possible that unmutated CLL cells derive from MZ B cells (or more likely a special subset thereof), given the recent relationships outlined at least in the mouse between B1 and MZ B cells.¹⁵

Mutated chronic lymphocytic leukemia

The progenitor of these neoplastic cells have to be selected among the B cells which utilize mutated VH/VL genes. Therefore, we have two options. This cell could be a memory cell that has exited from the GC and has initiated the transforming process or it is a MZ B cell that, following antigenic stimulation, has accumulated Ig V gene somatic mutations and subsequently has completed the transforming process. In both cases, accumulation of Ig V

gene somatic mutation should precede the transforming process, since unmutated clones would have a great advantage for their expansion compared to unmutated clones. Both hypotheses are compatible with the findings that CLL cells share GEP with normal memory B cells. At present it is difficult to choose between the hypotheses, given the relative scanty information on both the Ig V gene repertoire of MZ B cells and the memory B cells to be compared to that of CLL B cells.

References

1. Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukaemia. *New Engl J Med* 2005;352:804-15.
2. Chiorazzi N, Ferrarini M. B cell chronic lymphocytic leukemia: lessons learned from studies of the B cell antigen receptor. *Annu Rev Immunol* 2003;21:841-94.
3. Stevenson FK, Caligaris-Cappio F. Chronic lymphocytic leukaemia: revelations from the B-cell receptor. *Blood* 2004;103:4389-95. Review.
4. Morabito F, Mangiola M, Rapezzi D, et al. Expression of CD10 by B chronic lymphocytic leukemia cells undergoing apoptosis in vivo and in vitro. *Haematologica* 2003; 88:86-73.
5. Damle R, Batliwalla F, Ghiotto F, et al. Telomere length and telomerase activity delineate distinctive replicative features of the B/CLL subgroups defined by immunoglobulin V gene mutations. *Blood* 2004;103: 375-82.
6. Chiorazzi N. Cell proliferation and death: forgotten features of CLL B-cells. *Best Pract Res Clin Hematol* 2007;20:399-413.
7. Fais F, Ghiotto F, Hashimoto S, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. *J Clin Invest* 1998;102:1515-25.
8. Ghiotto F, Fais F, Valetto A, et al. Remarkably similar antigen receptors among a subset of patients with chronic lymphocytic leukemia. *J Clin Invest* 2004;113:1008-16.
9. Messmer B, Albesiano E, Efremov D, et al. Multiple distinct sets of stereotyped antigen receptors indicate a role for antigen in promoting chronic lymphocytic leukemia. *J Exp Med* 2004;200:519-25.
10. Widhopf GF, Rassenti LZ, Toy TL, et al. Chronic lymphocytic leukemia B cells of more than 1% of patients express virtually identical immunoglobulins. *Blood* 2004;104:2499-504.
11. Murray F, Darzentas N, Hadzidimitriou A, et al. Stereotyped patterns of somatic hypermutation in subsets of patients with chronic lymphocytic leukemia: implications for the role of antigen selection in leukemogenesis. *Blood* 2008;111:1524-33.
12. Klein U, Tu Y, Stolovitzky GA, 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.
13. Hervé M, Xu K, Ng YS, et al. Unmutated and mutated chronic lymphocytic leukemias derive from self-reactive B cell precursors despite expressing different antibody reactivity. *J Clin Invest* 2005;115:1636-43.
14. Hardy RR. B-1 B cell development. *J Immunol* 2006; 177:2749-54.
15. Martin F, Kearney JF. Marginal-zone B cells. *Nat Rev Immunol* 2002;2:323-35.