B-CHRONIC lymphocytic leukemia (B-CLL) is the most common leukemia in the western world. It is characterized by the relentless accumulation of mature CD5+ B cells (B1 B cells, according to the new nomenclature) and by a remarkably high frequency of autoimmune phenomena. For these reasons, B-CLL has been widely used as a model for the study of lymphoproliferative disorders and has given a great impulse to the study of the role of CD5+ B cells in autoimmunity.

From these studies CD5+ B-CLL cells have emerged as a crossroad between malignant and autoreactive B cells. Enough information is now available to put the unique biological and clinical features of B-CLL into a unifying perspective. The essence of the problem to be reviewed is three-fold. First, CD5+ B-CLL B cells express low to undetectable amounts of surface immunoglobulins (sIg)\(^8\). Second, B-CLL B cells accumulate in the G0 phase of the cell cycle.\(^6^6\) Third, the pathogenic autoantibodies (Abs), detectable in almost 30% of the patients, are usually polyclonal and target-restricted.\(^3^7\) They are directed against hematopoietic antigens expressed on the surface of red blood cells and, more rarely, of platelets and may lead to severe autoimmune hemolytic anemia and/or thrombocytopenia.\(^3^7\) These features led us to ask which is the actual normal counterpart of B-CLL B lymphocyte and how the molecular events that result in the malignant phenotype relate to the development of autoimmune manifestations.

THE QUEST FOR THE NORMAL COUNTERPART OF B-CLL

The quest for the normal counterpart of B-CLL has been focused on CD5+ normal B cells, but mantle zone (MZ) B lymphocytes, peripheral blood, and spleen B cells (B2 B cells)\(^7\) have also been analyzed.\(^8^12\) Our aim was to understand whether phenotypic features and functional properties might help in tracing back the cellular origin of B-CLL.

A number of cellular and molecular similarities between B-CLL and normal CD5+ B cells have emerged through these studies (Table 1). It has been noticed that monoclonal polyreactive IgM autoAbs, frequently of the rheumatoid factor (RF) type, are detectable both on the cell surface and in the serum of patients with B-CLL.\(^13^15\) In more than half of the CLL patients, the leukemic cells can be stimulated in vitro to secrete monoclonal polyreactive Abs.\(^16\) These findings closely match the observation that the function of CD5+ normal B cells is to produce polyreactive, low-affinity natural autoAbs.\(^15^17^18\) Interestingly, both normal and malignant CD5+ B cells produce polyreactive autoAbs in the absence of extensive somatic hypermutation.\(^19^24\) As the process of Ig gene somatic mutation occurs in the germinal centers (GC) of secondary follicles,\(^25\) its absence can be taken as a marker of the differentiative and functional level of the cell population analyzed. Also, both normal and malignant CD5+ B cells are skewed toward the use of the same restricted repertoire of nonmutated Ig V genes\(^21^26\) and share cross-reactive idiotypes (CRI).\(^27\) A number of other similarities between normal and malignant CD5+ B cells, like the ability to form rosettes with mouse erythrocytes, as well as the expression of myelomonocytic antigens and of low levels of CD20\(^28^29^30\) are also known, but their functional significance is still unclear.

The differences between normal and malignant CD5+ B cells are impressive (Table 1). First of all, normal CD5+ B cells do not have the low to undetectable levels of sIg that are so typical of malignant B-CLL. Next, B-CLL cells show an asynchrony between the phenotype, which is activated, as witnessed by the expression of several B-cell activation associated antigens (Ag),\(^12^33\) and the position within the cell cycle, which is fully resting as documented both by classic kinetic studies\(^6\) and by the negativity of c-myc protein expression.\(^34\) On the contrary, the phenotypic features of normal CD5+B cells are synchronous with the cell cycle phases.\(^12^29^31^32^33\) and c-myc is regularly expressed (unpublished results, August 1994). B-CLL cells also have unusual adhesive properties that reflect a likewise unusual cytoskeleton organization which, among normal lymphohemopoietic cells, is shared only by the monocyte-macrophage lineage.\(^15\) Further, it is extremely difficult to transform B-CLL cells...
with Epstein-Barr virus (EBV) even if they express the EBV receptor.\textsuperscript{26} This difficulty is remarkably absent in normal CD5\textsuperscript{+} B, where EBV has been used as a tool to probe their Ig gene repertoire and Ab production.\textsuperscript{37} Another difference observed is the B-CLL reduced response to the signals that conventionally induce the proliferation of normal B cells. A large number of patients with B-CLL have a defective signal transduction via Ag receptor as they fail to proliferate in response to anti-\(\mu\) Abs or anti-\(\mu\) and cytokines.\textsuperscript{8,7} In a few cases, also have a defective Ca\textsuperscript{2+} response coupled with an altered pattern of protein tyrosine phosphorylation.\textsuperscript{27}

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<td><strong>Production of polyreactive autoAbs</strong></td>
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ANERGIC B LYMPHOCYTES AND B-CLL CELLS: A COMPARISON

Anergy, ie the induction of a functionally silent state following the interaction of slg with self Ag, is a major mechanism of self-tolerance in the B-cell compartment. CD5\textsuperscript{+} (B1)\textsuperscript{+} and CD5\textsuperscript{-} (B2)\textsuperscript{+} B lymphocytes appear to obey different rules for the induction of tolerance to autoAg,\textsuperscript{27} even if the precise molecular mechanisms are still unknown. Normal B cells anergized by the exposure to self Ag in soluble form downregulate the expression of slgM.\textsuperscript{52-54} Therefore, among normal B lymphocytes, only anergic cells have levels of slgM that are as low as those of B-CLL cells. Tolerant autoreactive murine B cells phenotypically resemble MZ B lymphocytes\textsuperscript{32-33} and share with B-CLL cells the inability to respond to BCR ligation and a severely impaired BCR-mediated PTK activation.\textsuperscript{40,55} The block in the BCR-mediated pathway can be overcome by stimuli delivered by T helper cells and mediated by CD40 and cytokines.\textsuperscript{56}

B-CLL NORMAL COUNTERPART CANDIDATE: AN OPERATIONAL DEFINITION

Few conclusions on the nature of the B-CLL normal counterpart candidate can be safely drawn from a multitude of experimental observations. First, it is a normal B cell that expresses CD5. Normal CD5\textsuperscript{+} B cells are localized at the edge of GC\textsuperscript{28} and within the MZ of secondary lymphoid follicles, where normal B cells that express the CRI most frequently represent on the surface of malignant B-CLL cells are also confined.\textsuperscript{27} Second, it is likely a preswitch, pre-GC element, as the vast majority of B-CLL cells express either slgM or slgD and slgD, but only exceptionally slgG,\textsuperscript{37} and have not undergone somatic mutations. Third, it is devoted to the production of polyreactive, low-affinity autoAbs frequently of the RF type. Fourth, it has a number of features typical of anergic B cells, including low levels of slgM,\textsuperscript{52-54} a MZ-like phenotype,\textsuperscript{32,33} and the inability to respond to BCR ligation.\textsuperscript{40,55} On this basis, the B-CLL normal counterpart may be operationally defined as an anergic self-reactive CD5\textsuperscript{+} B cell committed to produce natural autoAbs.

By using this definition, we may put together the different pieces of the puzzle and propose that the target of the transforming event(s) that leads to B-CLL is a CD5\textsuperscript{+} antiself B cell that has become anergic after interaction with a self Ag and, therefore, expresses low levels of slg and has not yet undergone the GC-based processes of isotype switching and somatic mutation (Fig 1, upper panel). This target cell retains its original antiself reactivity after malignant transformation and may even use it as a survival kit that, preventing apoptosis, leads to the expansion of the malignant B-cell population. A human B-CLL has been described whose cells were characterized by the production of pathogenic RF and by an unusually rapid cell death after being placed in tissue culture: the in vitro addition of aggregated human IgG inhibited apoptosis and significantly enhanced the cell survival.\textsuperscript{27} The high proportion of B-CLL cases that express autoAbs on...
the cell surface\textsuperscript{13-15} induces speculation that the mechanism described\textsuperscript{58} may not be simply anecdotal, but rather have a more generalized significance. The transformed CD5\textsuperscript{+} anti-self B cell would secondarily acquire a number of properties, including failure to respond to EBV, low levels of antipoter activity, defective Ca\textsuperscript{++} response, poor response to mitogens, high levels of Bel-2,\textsuperscript{47-48} and low levels of Fas\textsuperscript{59} that ensure the unusual lack of kinetic flexibility typical of B-CLL and favor the accumulation of B-CLL cells in the G0 phase of the cell cycle (Fig 1, lower panel).

B-CLL CELLS AS ANTIGEN-PRESENTING CELLS (APC): A CLUE TO THE PRODUCTION OF POLYCLONAL PATHOGENIC AUTOAbs

If B-CLL is a monoclonal accumulation of anergic CD5\textsuperscript{+} anti-self B cells devoted to the production of polyreactive, natural autoAbs, why the autoimmune phenomena that mark its clinical course are caused by polyclonal high affinity autoAbs and why the pathogenic autoAbs have a restricted target antigen?

The operational definition of B-CLL normal counterpart may be used to analyze several experimental data and provide a coherent explanation for these problems. The policlonality of autoAbs by itself speaks against the possibility that they are produced by malignant cells and underlines the role of nonclonal residual normal B cells. Normal CD5\textsuperscript{+} B cells produce low avidity, polyreactive autoAbs predominantly of the IgM class (naturally occurring autoAbs), while the pathogenic autoAbs are usually of IgG class, monoreactive, and of high affinity.\textsuperscript{27-28} The absence of somatic mutations in the process of antigen-presenting cells (APC) and T cells to generate antise-self autoAbs. The risk is inherent in the mechanisms that lead to the generation of Ab diversity.\textsuperscript{25,61,62} It would be sufficient for a B cell producing polyreactive, low-avidity Abs to self Ag to undergo the process of somatic hypermutation that occurs in GC in the presence of antigen-presenting cells (APC) and T cells to generate high-avidity autoAbs.\textsuperscript{25}

The possibility that B-CLL cells may become, in an appropriate setting, efficient APC and present restricted hemato-

![Diagram](Image)

**Fig 1.** Scheme of the events presumably involved in the malignant transformation of the candidate B-CLL normal counterpart.

polytic self-Ag to normal residual B cells may provide a clue to understand the production of polyclonal pathogenic autoAbs. This possibility is based on a number of observations. First, it is now well established that the generation and amplification of immune responses is critically dependent on the interactions of costimulatory molecules.\textsuperscript{53} The best known examples are the CD40-CD40 Ligand (L) interaction that allows B cells to respond to T cells\textsuperscript{26} and the interaction of molecules of the B7 family expressed by APC with their counterreceptor CD28 expressed on the surface of T cells,\textsuperscript{65} which leads to T-cell activation and cytokine gene expression. The B7 family plays a central role in transplantation tolerance and in tumor immunity\textsuperscript{66}: its two best defined members are B7-1, designated as CD80 and B7-2, designated as CD86.\textsuperscript{65} It is pertinent to this discussion the finding that B7 costimulatory molecules are expressed in vitro by activated B cells\textsuperscript{65} and in vivo by malignant B cells.\textsuperscript{68} The second observation is that, while normal B cells are very effective APC,\textsuperscript{69} normal anergic B cells have a reduced ability to process and present Ag,\textsuperscript{71} but remain sensitive to T helper signals.\textsuperscript{64} The half-life of anergic B cells is short, but the anergic state may be reversed on contact with activated T cells during chronic Ag exposure.\textsuperscript{65,66} The inability of anergic B cells as APC is, at least partly, explained by the low levels of sIg and by the suboptimal expression of costimulatory molecules like CD86.\textsuperscript{71} Interestingly, weakly sIg+ B-CLL cells also have low levels of expression of CD80 and CD86 and likewise fail to present soluble and allo-antigen.\textsuperscript{70,71} In vitro experiments have shown that the stimulation of CD40 on the membrane of B-CLL cells by the T cell-expressed CD40L upregulates the expression of CD80 and CD86 molecules, thereby transforming anergic B-CLL cells into very effective APC.\textsuperscript{70} The APC function of B-CLL cells may be further enhanced by another set of T cell/B cell cross talks, represented by the CD27-CD70 interactions.\textsuperscript{71}

Taken together, the above-discussed data suggest that, in vivo, B-CLL cells may be activated via the CD40-CD40L and (perhaps also) the CD27-CD70 interactions to become fully capable APC. It is easy to envisage that a very appropriate environment for these interactions to successfully occur is the spleen. As a major function of the spleen is the sequestration and removal of senescent and abnormal blood cells, it follows that B-CLL cells in the spleen are incessantly exposed to a number of anucleated effete cells (red blood cells and platelets) that are removed from the circulation by the mononuclear phagocyte system. It is not unreasonable to postulate (Fig 2) that, in splenic microenvironment, B-CLL cells may come in contact with soluble forms of CD80 and CD86 molecules and/or platelet membrane structures and, upon activation by T cells, acquire the ability of presenting these membrane degradation products (self-Ag) to residual normal B cells. This possibility is well in keeping with the observation that anergic B cells from double transgenic mice express B7 in presence of powerful stimuli such as an antigen anchored in a cell membrane.\textsuperscript{59} In this setting, B-CLL residual normal B cells would be driven to produce the high-affinity polyclonal autoAbs with restricted specificity and pathogenic properties that cause autoimmune hemolytic anemia and/or thrombocytopenia in B-CLL. As the effete cells removed in
the spleen are anucleated, this scenario may explain not only the red blood cell- and platelet-restricted antiself reactivity, but also the findings that the autoAbs that characterize systemic autoimmune diseases, like antinuclear and anti-DNA antibodies, are virtually absent in B-CLL and that the association between B-CLL and systemic lupus erythematosus (SLE) is, at best, exceptional.\(^7\)

**WHICH B-CELL SUBSET IS INVOLVED IN THE PRODUCTION OF POLYCLONAL AUTOABS IN B-CLL?**

At present, it cannot be defined whether the polyclonal autoAb-producing cells belong to the CD5\(^+\) (B1)\(^2\) or the CD5\(^-\) (B2)\(^2\) cell subset. As CD5\(^+\) B cell \(V_{H}\) and \(V_{L}\) genes are mainly in germ-line configuration,\(^3\) they are believed to act essentially in primary responses with little GC formation and restricted somatic mutations. This would favor the possibility that CD5\(^+\) (B2) B cells are responsible for the production of polyclonal autoAbs in B-CLL. Still, it is possible that a proportion of CD5\(^-\) B cells enter GC, switch from IgM to IgG\(^+\) B cells and undergo the somatic mutations that lead to the production of high-affinity Abs.\(^12,24,25\) It would be tempting to think that in B-CLL the monoclonal malignant cells that have acquired an APC role may fish in a pool of normal residual CD5\(^-\) B lymphocytes and persuade some nonmalignant CD5\(^+\) B cells to enter the high affinity autoAb-producing pathway. This possibility would be in keeping with the observation that a polyclonal expansion of CD5\(^-\) B cells is observed in systemic autoimmune diseases like rheumatoid arthritis, Sjögren syndrome, systemic sclerosis, and, occasionally, SLE.\(^12,33\) Also, a genetic influence on the levels of circulating normal CD5\(^-\) B lymphocytes has been shown to exist,\(^74\) and it will be of interest to prospectively evaluate whether high levels of normal circulating CD5\(^-\) B cells may be a “predisposing” factor to the development of B-CLL or of systemic autoimmune diseases. Nevertheless, every explanation has to cope with the fact that interleukin (IL)-1 and IL-2, the cytokines that control the progression of B lymphocytes through GC, are able to downregulate CD5 expression in purified CD5\(^-\) B cells.\(^75\) Therefore, the high-affinity autoAb-producing B lymphocytes, irrespective of their derivation, would appear as CD5\(^-\) B cells.

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**REFERENCES**


23. Hummel M, Tamariu J, Kalvelage B, Stein H: Mantle cell (previously centrocytic) lymphomas express VH genes with or very little somatic mutations like the physiologic cells of the follicle nantic. Blood 84:403, 1994


73. Lugassy G, Lishner M, Polliack A: Systemic lupus erythematosus and chronic lymphocytic leukemia: Rare coexistence in three patients, with comments on pathogenesis. Leuk Lymphoma 8:243, 1992
75. Caligaris-Cappio F, Riva M, Tesio L, Schenu M, Gaidano GL, Bergui L: Human normal CD5+ B lymphocytes can be induced to differentiate to CD5+ B lymphocytes with germinal center cell features. Blood 73:1259, 1989
B-chronic lymphocytic leukemia: a malignancy of anti-self B cells

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