Facts and Speculations on the Onset and Clonal Expansion of Chronic Lymphocytic Leukemia Cells

Manlio Ferrarini, MD1, Daniela de Totero, PhD2, Monica Colombo, PhD1 and Giovanna Cutrona3, PhD

Abstract— In this review, we describe the major phenotypic and functional features of CLL cells. Moreover, we discuss the interactions with the microenvironment required for clonal survival and expansion. These interactions include signals delivered directly via cell-cell contact with cells like T cells, monocytes/macrophages and nurse-like cells and those delivered by cytokines and chemokines. These interactions may facilitate clonal expansion, but also may serve to limit the clonal size, a finding which can be exploited for therapeutic strategies. Signals delivered through their B cell Receptor (BcR) can also be instrumental for CLL cell clonal survival and expansion; the BcR specificity of the single clones may dictate disease aggressiveness. Genetic lesions, which are amply documented in CLL, and are constituted by chromosomal deletions or duplications and by inactivating gene mutations, may be particularly relevant in the late stages of clonal expansion as well in the control of response to therapy. In contrast, lesions causing deregulations of miRNA, which control a number of cellular functions, may be involved in the pathogenesis of the early CLL steps.

Keywords — Chronic Lymphocytic Leukemia, microenvironment, B cells, B cell receptor, microRNA.

I. INTRODUCTION

CLL has been traditionally considered as caused by the relentless accumulation of small, possibly immune-incompetent lymphocytes in the peripheral lymphoid organs and in blood[1]. New refinements have been subsequently added to this old concept, including the notion that the leukemic cells were B cells because of their expression of surface membrane immunoglobulin (sIg) and that the cell expansion was monoclonal as documented by the monotypic restriction of sIg for K or lambda light chains[2-4]. An additional refinement of these notions also was represented by the hypothesis that CLL cells had an impaired apoptosis, which facilitated their accumulation[5].

A number of more recent observations have generated problems with the initial model. For example, in vivo labeling studies, with deuterated water, have demonstrated that CLL cells have a rapid turn-over characterized by cell proliferation and cell death[6]. This turn-over may involve up to 1% of the clone daily, and is particularly pronounced in those cases in whom the disease is clinically more aggressive and whose cells express unfavorable prognostic markers (see below). CLL cells have shorter than normal telomeres and higher telomerase activity and these features are particularly evident in the cells from more aggressive cases[7-11]. Since telomeres shorten with cell proliferation, this observation suggests again a rather rapid cell turnover. Finally, highly purified CLL cells undergo spontaneous apoptosis after short periods in culture and do not exhibit major apoptotic defects although, conditions of resistance to apoptosis by CLL cells have been documented as discussed later. Collectively, the data indicate a rapid renewal of the leukemic clone in vivo, possibly related to a combination of cell proliferation and death.

These new concepts raise several questions related to the factors promoting cell proliferation and preventing apoptosis, to the interaction between CLL cells and stromal cells[12-16], and also to the nature of the genetic lesions which facilitate clonal expansion[17, 18]. None of these issues has so far found a complete answer, although the emerging data from the studies of the last ten years begin to provide a more coherent picture of the mechanisms involved in both disease onset and progression.

Support provided by the microenvironment

Purified CLL cells die rapidly when placed in culture unless other cells (or supernatant thereof) are concomitantly present. This very simple co-culture methodology was employed to clarify the interactions between CLL cells and stromal cells. Bone marrow stromal cells were the first stromal cells found to be capable of preventing CLL cells apoptosis via Beta 1/2 integrins [19]. Moreover, CLL cell spontaneous apoptosis is prevented in vitro by the addition of fibroblasts transfected with CD40L [20], of activated T cells[21-24], and of activated cells from the monocyte/macrophage lineage[15, 25]. Nurse-like cells, a particular type of stromal cells present in vivo in spleen
and lymphoid tissues, and differentiating in vitro from the monocyte component of peripheral blood of CLL patients, are very efficient in sustaining CLL cell survival [26, 27]. A detailed description of the interactions occurring between CLL cells and accessory cells goes beyond the scope of this article, but some important notions should be pointed out. First, the supporting effect of stroma may be exerted directly by the interaction with CLL cells or may be mediated by soluble factors[26, 28-31]. These can be cytokines or chemokines released by the accessory cells and for which CLL cells have specific receptors[31-33]. These chemokine not only serve to promote CLL cell survival/proliferation, but also may possess a broader regulatory function as documented perhaps by the opposite effects of IL 15 and IL21. IL15 prevents CLL cell apoptosis in vitro and induces cell proliferation, while IL21 inhibits cell proliferation and induces cell apoptosis[34-36]. These negative regulatory functions of IL21 are currently exploited in therapeutic strategies[37, 38]. Second, the expression of cytokine/chemokine receptors can represent markers of particular CLL clones or subclones. For example, IL21R is preferentially expressed by the clones from patient with more indolent clinical disease, a finding that is in line with the functional data described above[34]. The cells which express high levels of CXCR4 (and low CD5 levels) within a given leukemic clone are less activated and less prone to proliferation than the remaining cells with low CXCR4 (and high CD5 levels)[39] but have the property of growing easily in immunodeficient mice[40]. Third, the close interaction occurring between leukemic cells and stroma indicates that CLL cell proliferation cannot occur everywhere in lymphoid organs and blood, but requires specialized sites, a consideration consistent with the observation that there are proliferating centers in lymphoid tissues where there is accumulation of both accessory cells and CLL cells. The leukemic cells in these structures express more abundant activation markers and anti-apoptotic molecules[41-43]. Therefore, it is likely that, while clonal expansion occurs primarily in these sites, clonal loss takes place in other sites like peripheral blood, where cellular interactions are prevented. Finally, the close interactions occurring during clonal expansion may indicate new strategies for therapy since cytokines/chemokines, their receptors or molecules involved in cellular interactions can become suitable targets for biological therapies[15, 44, 45]. Fig 1 reports a schematic view of these interactions.

---

**Fig. 1. Schematic representation of the interactions occurring between CLL cells and the micro-environment.** The major interactions which involve both cells and cytokines/chemokines are depicted. Courtesy of Dr. S. Ferrini.

**Surface Immunoglobulin (sIg)**

The expression of sIg by CLL cells was described in the early seventies of the last century[2-4, 46], although the possibility that sIg (now currently referred to as B cell receptor or BcR) could play a role in clonal survival/ expansion was considered more lately. The first indication that BcR could have a function in the survival of B cells came from observations on normal murine B cells. Surface IgM (or CD79a, a molecule involved in the BcR signal transduction pathway) was conditionally inactivated in the mature peripheral B cells of genetically engineered mice. These mice lost progressively their mature B cell compartment[47, 48]. This finding indicated that the BcR exerted a trophic function possibly by delivering “sub-threshold” stimulatory signals to the B cells[49, 50]. These observations also explained why mature B cells from the large majority of lymphoproliferative disorders continue to express a fully assembled, functional BcR, which likely exerts a trophic function. Reed-Sternberg cells of Hodking lymphomas represent a remarkable exception[51], suggesting the interesting hypothesis that the acquisition of a new pathway for survival independent of BcR signaling is part of the transformation process in this disease. The idea that BcR stimulation by antigen could be involved in clonal expansion in CLL came later when gene sequence studies became available and demonstrated correlations between IGHV/IGLV gene features of the single CLL clones and clinical course and outcome of the patient[52-55]. First of all, it was found that CLL clones could be subdivided into two distinct groups, i.e. clones exhibiting somatically mutated IGHV genes and clones which did not have these somatic mutations[56]. These findings introduced concepts of biological relevance since indicated that the patients carrying cells with somatic mutations had CLL clones (M-CLL) probably originated by antigen-experienced B cells[57-59]. In fact, B cells activated by specific antigens enter the GC where
they undergo a process of somatic hypermutation (SHM) in their rearranged IGHV and IGLV gene segments[60]. As a consequence, the cells express new BcR and only those cells that have developed high affinity BcR to the stimulating antigen are further selected to become memory cells or antibody secreting plasma cells. SHM may also occur outside the GC in the Marginal Zone (MZ) of the spleen or in the MZ-like areas of other lymphoid tissues, although to a lesser extent[61]. Nevertheless, antigenic stimulation is required for SHM to occur also in these sites[13, 62]. The antigenic stimulation of M-CLL clones was likely to occur prior, during and also perhaps following leukemogenesis[13]. The description of M and U CLL has clinical relevance since led also to the definition of two distinct groups of patients. U-CLL patients have a rapidly progressing clinical course and often a dire outcome, while M-CLL patients exhibit an indolent disease[53, 54]. These findings also confirmed previous clinical observations showing that CLL patients can be subdivided into two groups based upon clinical course and outcome and provided the means to distinguish the patients from the two groups.

The above findings pose a number of questions. For example, what is the nature and origin of the cells from U-CLL cases? Are these cells virgin B cells or else antigen-experienced B cells? Given their expression of sIg, these cells are clearly mature peripheral B cells, although the issue of their prior history, including the possibility of antigen encounters is somewhat debated[18, 59, 63]. The answer to this question depends in part upon the experimental approach taken to solve the problem. In a number of studies, gene expression profiles (GEP) or miR expression profiles of CLL cells were compared to those of subsets of normal B cells purified from splenic or tonsil tissues or from peripheral blood. In general, U-CLL and M-CLL exhibit very similar GEP or miR expression profiles, although a set of genes or of miR capable of distinguishing between the two groups can be identified[57, 64, 65]. Most of the comparisons with normal B cell subsets indicated that the signatures of CLL cells were more similar to those observed for memory B cells and MZ or MZ like-B cells. These subsets of B cells, which seed in the splenic MZ or in the MZ-like areas of tonsils, exhibit very similar signatures. However, other investigators observed strong GEP similarities between U-CLL cells and the cells from peripheral blood characterized by the presence of CD5 and the absence of CD 27. Conversely, M-CLL cells exhibited GEP similarities with CD27-positive, CD5-positive circulating B cells[66]. The interpretation provided by these investigators was that U-CLL originated from virgin B cells whereas M-CLL originated from memory B cells.

Another approach to the issue was that of analyzing the IGHV and IGLV gene repertoire of U-CLL clones and to compare it with that utilized by normal B cells. The rational behind this approach was that, if the gene repertoire utilized by a sufficient number of CLL clones was similar of that of normal, virgin B cells, then the observation would have been compatible with a CLL cell origin from virgin B cells. If, however, a skewed usage of IGHV or IGLV genes or of a combination thereof was to be found, then this would have provided evidence that antigenic stimulation, occurring prior or in the course of leukemogenesis, was responsible for the imbalanced repertoire observed and that U-CLL cells were indeed antigen-experienced B cells[52]. In connection with this it is worth recalling that antigenic stimulation by particular antigens such as, for instance, bacterial polysaccharides occurring in the absence of T cells, does not generally lead to SHM[52]. Indeed, several studies have demonstrated that in U-CLL, and in part also in M-CLL, clones there is a preferential utilization of certain IGHV/IGLV genes compared to normal. Moreover, in approximately 30% of U-CLL cases there is frequent utilization of stereotyped BCR, i.e. molecules that share similar CDR3 segments and are encoded for by the same IGHV/IGLV gene. CLL cases can be sub-grouped based upon the utilization of shared stereotyped receptors[67-72]. Together, these findings suggest not only that antigen stimulation may be responsible for the differences of CLL and normal B cell repertoire, but also that this stimulation is operated by special antigens given the repertoire restriction of CLL cells and the utilization of groups of stereotyped receptors.

A number of studies were addressed to the issue of the specificity of the CLL cell BcR. Initially, it was demonstrated that a number of CLL cases utilized BcR with polyclonal (auto)reactivity, i.e. the BcR had a broad reactivity for several antigens such as IgG molecules, platelet antigens, double or single stranded DNA etc)[73-78]. These BcR were primarily utilized by U-CLL clones. More recently, antigens expressed by apoptotic cells[78-80], internal epitopes of the BcR itself[81]and fungal antigens were included in the list[82](see table 1). The observation that antigens constantly present in vivo such as autoantigens can bind to the CLL BcR suggests that a continuous antigenic stimulation may serve as promoting agent for clonal expansion[83]. Indeed, this concept is corroborated by several observations. First, CLL cells express typical B cell activation markers and U-CLL are characterized by the expression of a larger number of these markers and of markers indicating a more recent activation[58]. Since they utilize more frequently autoreactive (polyspecific) BcR, it can be inferred that this more pronounced activation can at least in part be related to a continuous and possibly more robust antigenic stimulation. Interestingly, the preferential expression of certain markers such as CD38, ZAP-70 or CD49d by U-CLL can be used to distinguish this group of cases[7, 84-86]. Moreover, the expression of these markers by the leukemic cells has a predictive value for progression and clinical outcome. Second, there is a broad correlation between clinical course and BcR specificity. CLL cases sharing the same subsets of stereotyped BcR also have similar clinical courses. Moreover, CLL cells equipped with BcR with polyspecific reactivity have a worst clinical course than those without these BcR[87].

Since they utilize more frequently autoreactive (polyspecific) BcR, it can be inferred that this more pronounced activation can at least in part be related to a continuous and possibly more robust antigenic stimulation. Interestingly, the preferential expression of certain markers such as CD38, ZAP-70 or CD49d by U-CLL can be used to distinguish this group of cases[7, 84-86]. Moreover, the expression of these markers by the leukemic cells has a predictive value for progression and clinical outcome. Second, there is a broad correlation between clinical course and BcR specificity. CLL cases sharing the same subsets of stereotyped BcR also have similar clinical courses. Moreover, CLL cells equipped with BcR with polyspecific reactivity have a worst clinical course than those without these BcR[87].
potential contribution of genetic lesions to clonal expansion

Antigenic stimulation alone is unlikely to sustain clonal expansion even with the support of cytokines and accessory cells. Conceivably, genetic lesions characterizing the CLL cells have to contribute to both cell survival and clonal expansion. Several cytogenetic lesions have been described in CLL including 17p, 11q deletions and trisomy 12[93-95]. These lesions result in the loss of p53 (17p- ) and ATM (11q-) genes, which are important in the control of cell apoptotic capacities. These lesions, however, are evident in a substantial number of cases at the advanced clinical stages, but are much less frequent in the early cases in which the lesions are confined often to a minority of cells. Therefore, although these lesions are relevant for disease progression, response to therapy and outcome may not be of pathogenic importance for the origin of the disease[96]. Likewise, inactivating mutations of other genes such as NOTCH1, BIRC3, SF3B1, which are relevant in the control of cell survival and apoptosis, are detected in a substantial proportion of cases at advanced stages, but are less frequent at the disease onset, again suggesting the importance of these genes for disease progression and outcome, but not for the pathogenesis of the disease[97-102]. 13q- deletion represents a quite remarkable exception among the recurrent cytogenetic lesions of CLL because it is detected in a substantial number of cases (up to 60-70%) in the early stages including monoclonal B cell lymphocytosis[103-105]. The genes involved by the deletion have been somewhat mysterious for long time, until Carlo Croce and his collaborators discovered that the deletion of Leu2 pseudogene, which is comprised in the 13q- deletion, leads to the concomitant loss of the DNA segments encoding two microRNA (miR) miR15a and miR 16-1, which lie within the Leu2 gene[17]. miRs are small, non coding RNAs which regulate protein synthesis by binding to mRNA and altering its transcription and/or inducing its degradation. Inactivating mutations of a single miR allele are recessive, i.e. miRs behave as tumor suppressor genes.

**Table 1: Major specificities described for the BcR of CLL cells.**

<table>
<thead>
<tr>
<th>Polyspecific antibodies reacting with self-antigens such as IgG molecules, platelet antigens, DNA, etc. (observed mainly in U-CLL)[73-78]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auto-antigens express by apoptotic cells (mainly U-CLL)[78-80]</td>
</tr>
<tr>
<td>Fungal antigens (M-CLL)[82]</td>
</tr>
<tr>
<td>Internal epitopes of the BcR itself[81]</td>
</tr>
</tbody>
</table>

Finally, patients, whose cells have a functioning BcR-dependent signal-transducing pathway, have a worst clinical course than patients with impairment in this pathway[88] (see table 2).

In conclusion, there is now evidence that leukemic cells from both the M and U-CLL cases may be antigen experienced B cells, based on evidence coming from different experimental approaches, although different opinions have been reported by certain investigators. The notion that the activation of the BcR-dependent signal-transducing pathway may have a role in clonal expansion has practical consequences. For example, inhibitors of the Bruton tyrosin kinase (TK), a specific TK of the BcR-dependent pathway has a powerful therapeutic effect in reducing CLL clonal size. Likewise, inhibitors of the PI3K, which also is involved in the BcR-dependent pathway, in addition to other signal transducing pathways, has a powerful therapeutic effect. Administration of these TK inhibitors results in a raise of circulating CLL cells concomitantly to lymph nodes shrinking[89-92] This would indicate that the CLL cells are somehow anchored to the accessory cells in the proliferating centers via BcR and/or other surface adhesion molecules and that the blocking of their signal transducing function causes the exit from the proliferating centers and the transfer to the periphery of CLL cells where they possibly undergo a slow apoptotic process. All of these phenomena are compatible with the data of the studies performed with deuterated water alluded to above implying a rapid CLL cell turn-over consequent to proliferation and death of the CLL cells[6].

**Table 2. Differences in CLL cells features supporting a role of BcR stimulation in disease progression.**

<table>
<thead>
<tr>
<th>Progressor</th>
<th>Non progressors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutated IGHV</td>
<td>unmutated IGHV</td>
</tr>
<tr>
<td>viable BcR-mediated pathway</td>
<td>non-viable BcR-mediated pathwy</td>
</tr>
<tr>
<td>sterotyped BcR *</td>
<td>non-stereotyped BcR</td>
</tr>
<tr>
<td>Presence of B-cell activation markers</td>
<td>Absence of B-cell activation markers</td>
</tr>
</tbody>
</table>

* Most cases whose cells utilize sterotyped receptors have an aggressive clinical course, although this is not a general rule

**Potential contribution of genetic lesions to clonal expansion**

Antigenic stimulation alone is unlikely to sustain clonal expansion even with the support of cytokines and accessory cells. Conceivably, genetic lesions characterizing the CLL cells have to contribute to both cell survival and clonal expansion. Several cytogenetic lesions have been described in CLL including 17p, 11q deletions and trisomy 12[93-95]. These lesions result in the loss of p53 (17p- ) and ATM (11q-) genes, which are important in the control of cell apoptotic capacities. These lesions, however, are evident in a substantial number of cases at the advanced clinical stages, but are much less frequent in the early cases in which the lesions are confined often to a minority of cells. Therefore, although these lesions are relevant for disease progression, response to therapy and outcome may not be of pathogenic importance for the origin of the disease[96]. Likewise, inactivating mutations of other genes such as NOTCH1, BIRC3, SF3B1, which are relevant in the control of cell survival and apoptosis, are detected in a substantial proportion of cases at advanced stages, but are less frequent at the disease onset, again suggesting the importance of these genes for disease progression and outcome, but not for the pathogenesis of the disease[97-102]. 13q- deletion represents a quite remarkable exception among the recurrent cytogenetic lesions of CLL because it is detected in a substantial number of cases (up to 60-70%) in the early stages including monoclonal B cell lymphocytosis[103-105]. The genes involved by the deletion have been somewhat mysterious for long time, until Carlo Croce and his collaborators discovered that the deletion of Leu2 pseudogene, which is comprised in the 13q- deletion, leads to the concomitant loss of the DNA segments encoding two microRNA (miR) miR15a and miR 16-1, which lie within the Leu2 gene[17]. miRs are small, non coding RNAs which regulate protein synthesis by binding to mRNA and altering its transcription and/or inducing its degradation. Inactivating mutations of a single miR allele are recessive, i.e. miRs behave as tumor suppressor genes.
Therefore, the most obvious consequences on the cell physiology appear when the lesions of the miRs encoding genes are bi-allelic, although a gene dosage effect can be observed in the cells with monoallelic deletions[106-109]. A number of observations indicate the importance of miR15a and of miR16-1 in CLL pathogenesis. Germ-line deletions of these miRs were detected in individuals with familial CLL [110]and similar genetic lesions are found in strains of New-Zealand mice, which are particularly prone to develop a CLL-like disease [111-113]. Moreover, genetically engineered mice, in which the Leu2 gene was deleted, together with the relative miRs encoding gene segments, proved particularly prone to develop a CLL-like disease[114]. Although the regulatory functions of miR 15A and miR 16-1 are not fully understood, it is of note that these two miRs participate in the negative regulation of the bcl-2 gene expression, which is involved in apoptosis control[115]. Thus, CLL cells lacking these two miRs have high levels of BCL-2 protein, which fall progressively upon transfection of either miR15a or miR16-1 in vitro, and the cells become more apoptosis prone (Cutrona G et al manuscript in preparation). Moreover, CLL cells transfected with these miRs virtually lose their capacity to grow in immunodeficient mice[113].

These observations on miR15a and miR16-1 have promoted further investigation. Indeed, miR expression profiles in CLL have demonstrated either significant up- or down-regulation of a number of miRs compared to normal B cells. Many differences remain significant when MZ or memory B cells, which have a miR signature most similar to that of CLL, are used for comparison [65]. Some of these differences correlate with disease progression and outcome possibly indicating a mechanistic role[110]. However, the causes leading to altered miR expression are not fully understood since some of them may be consequent to the constitutive activation status of the CLL cells mentioned above and others related to mutations in the DNA segments encoding the miRs. Clearly, the issue deserves additional studies, since the concept that lesions in the regulatory functions mediated by miR may be part of the mechanisms of disease is particularly appealing.

II. CONCLUSIONS

The above considerations lead to a rather general model of CLL pathogenesis. Genetic lesions, particularly in the miR encoding genes, may determine a dysregulation of the cell response to external stimuli. These may include those delivered by BcR interacting with antigens and particularly self-antigens and those provided by the microenvironment (i.e. cytokines and accessory cells). This collaboration between signals delivered by antigenic stimulation as well as by the microenvironment may determine clonal expansion and facilitate the accumulation of additional genetic lesions (e.g. 17p- or 11q-). This may represent the framework for a general working hypothesis in which one could insert more refined and specific pathogenetic mechanisms.

Several issues remain to be clarified. For example, a large fraction, although not the totality, of CLL cases exhibit the 13q-deletion. Does this observation imply that there are other deregulated miRs which facilitate the onset of these CLL or else does the pathogenesis of these cases follow a totally different mechanism? Moreover, the mechanisms leading to altered miR expression in CLL compared to normal should be elucidated particularly with the aim of clarifying whether underlying lesions of the encoding genes exist. This is particularly true considering the relative low number of mutations reported by next generation sequence studies investigating exon sequences[18, 116-118].

There are other issues related to the mechanisms of disease which deserve clarification. For example, does a stem cell–like compartment exist in the CLL clones? Cell fractionation studies, in which the cells were separated according to the expression of CXCR4 and CD5, demonstrated that that cells with high CXCR4 and low CD5 are less activated, but more prone to developing CLL into immune-deficient mice. However, is this a real stem cell –like compartment of cells capable of long term replication and clonal expansion on which the search for new genetic lesions should be focused? Or else these cells represent simply a functional status rather than a defined stem cell subset? In other words, are the two subsets defined by CXCR4 and CD5 expression interchangeable in the sense that one cell type can transform into the other and vice versa, depending upon the microenvironment and the external stimuli received? Along this line, one should also face the problem of defining the nature of the cell of origin of CLL. For example gene or miR expression profiles studies have provided results which differ depending upon different experimental approaches. In contrast, data on repertoire studies have provided more unequivocal answer since they suggest that both the cells from U- and M-CLL are antigen-experienced cells. The former cells are probably stimulated in the absence or very little T cell help, whereas for M-CLL cells antigenic stimulation likely occurred in the presence of accessory T cells. However, even this statement is open to several interpretations. What are the T cell independent cells responsible for U-CLL cell origin? Are they MZ B cells, or the human equivalent of murine B1 B cells or even another, still undefined subset of transitional B cells? And are the B cells originating M-CLL post germinal center B cells or MZ B cells that have undergone SHM outside GC? Finally, even following an unequivocal identification of the cell of CLL origin, one has to consider that this cell may represent the last step where transformation occurred. Indeed, if leukemogenesis is a multistep series of events, then many of these events may have taken place well before the differentiation stage where full blown transformation and final clonal expansion has occurred[59].

Another issue to be elucidated relates to the cells susceptibility to stimulation via BcR within the CLL clone. Are all of the cells within the CLL clone susceptible to stimulation by (auto)-antigens or else can only a fraction of the cells from a CLL clone expand in response to antigenic stimulation? In the latter case, the responding fraction would represent a sort of stem cell-like compartment of the clone[39, 119]. The issue may become even more complex when considering also the requirements for accessory cell help and cytokines.
Despite the many uncertainties still existing on the fine pathogenetic mechanisms, CLL has been progressively defined as a disease caused by the expansion of a B cell clone which is for great part dependent upon stromal and cytokine/chemokine help and antigenic stimulation. These concepts were initially considered rather unique and applicable only to CLL. However, based upon a number of further observations, investigators have now realized that some of these notions can be applied also to other low-grade lymphoproliferative disorders[120, 121]. In this respect, CLL may have paved the path towards a new approach to the study of lymphoproliferations.

References


Kolb JP. IL-21 as new therapy for CLL?, *Blood*, 2008, 111 (9), 4323-30. [54]


Lam KP, Kuhn R, Rajewsky K. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death, *Cell*, 1997, 90 (6), 1073-83. [66]


Hamblin TJ, Davis Z, Gardiner A, Ossier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia, *Blood*, 1999, 94 (6), 1848-54. [73]


Chiorazzi N, Ferrarini M. Cellular origin(s) of chronic lymphocytic leukemia: cautionary notes and additional considerations and possibilities, *Blood*, 2011, 117 (6), 1781-91. [78]


lymphocytic leukemias derive from self-reactive B cell precursors despite expressing different antibody reactivity, J Clin Invest, 2005,115 (6), 1636-43.


