# The Etiology of Chronic Lymphocytic Leukemia: Another Look at the Relationship Between B1 cells and CLL

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**Abstract:** Chronic lymphocytic leukemia (CLL) is a heterogeneous disease of unknown etiological origin. CLL is the only B cell malignancy where a characteristic chromosomal translocation is not involved in cancer initiation. Therefore, the cause of tumorigenesis in CLL patients and the type of cell that is transformed are two questions that have intrigued researchers for decades. However, evidence suggests the CLL cell may be derived from B-1 cells. These B-1 cells, thought to develop during neonatal maturation as a link between innate and adaptive immunity, share multiple phenotypic and genetic patterns with CLL cases. These include signaling molecules sensitivity and expression patterns, B-cell receptor (BCR) specificity, and a unique immune-modulatory phenotype. Through understanding the biological relevance of B-1 cells in immune development and regulation, we may further understand the molecular mechanisms underlying the complexity of CLL. With this understanding, we can provide more optimal care to patients based on their unique diagnosis and pathologic disease course. In this review, based on our current understanding of CLL cells and B1 cells we hypothesize that CLL cells are originated from B1 cells. Following are some of our rationale in deriving this hypothesis.

**Keywords:** Chronic lymphocytic leukemia, B1 cells, B regulatory cells, CLL etiology.

## INTRODUCTION

Chronic lymphocytic leukemia (CLL) is an extremely heterogeneous disease with a highly variable clinical course. Although many patients live and die with the disease, other patients experience an aggressive disease course and ultimately die from the cancer [1]. However, the mystery of CLL remains in the origin of the disease. What causes CLL tumorigenesis, and what cell type is transformed to become CLL? These two questions continue to plague researchers trying to understand this intriguing ailment. By understanding the origination and instigating factors leading to the development of CLL, we hope to elucidate the molecular mechanisms underlying the complexities of this heterogeneous disorder. In so doing, we may provide more optimal care for patients with CLL based on their unique diagnosis and pathologic disease course.

To begin unraveling the mystery of CLL's origin, the phenotype of CLL cells must be considered. CLL cells are characterized as monoclonal B cells expressing various B cell-associated markers, including the surface CD19 and CD23 markers [2]. While the B cell lineage of CLL cells is rarely disputed, the presence of many T cell-associated markers in CLL cells has raised questions as to the exact derivation of CLL cells. For example, the T cell-associated CD4, CD7, and CD8 markers have all been found in certain clinical cases of CLL [3, 4]. Additionally, T cell activation-associated molecule zeta-chain-associated protein kinase 70 (ZAP-70) is expressed in a subset of CLL patients with unmutated immunoglobulin variable (IgVH) region genes, and its expression corresponds to increased B-cell receptor (BCR) signaling and shorter time to treatment in patients [5, 6]. In fact, one of the primary markers used to separate and identify CLL cells out of samples of peripheral blood mononuclear cells is CD5, which is considered by many to be a pan-T cell marker [7].

Interestingly, although CD5 is considered a T-cell marker, certain subsets of B cells are known to express CD5. The primary subgroup of CD5+ B cells is the B-1 cell type; these cells are thought to develop primarily during neonatal maturation (and possibly within the adult bone marrow) and demonstrate some poly-specificity [8]. These B-1 cells serve as a link between the innate and adaptive immune system, developing *via* a mechanism independent of the B2/traditional developmental program of B cells. Additionally, these cells bear resemblance in function and characteristics to the marginal zone (MZ) subset of B cells (distinct from the follicular, or FO B cells) that may be involved in rapid T-independent or memory immune responses [8, 9]. Both B1 and MZ subsets of B cells express CD9 and the CD27 memory B-cell marker. In fact, CLL cells have also been characterized as expressing CD27, suggesting their progenitor has an antigen-experienced memory phenotype in at least a majority of CLL cases [10].

Undeniably, there are a plethora of possible progenitor cells for CLL. Many have suggested that CLL cells could, indeed, be derived from the B-1 cell lineage [11-15]. On the other hand, others believe that CLL may have an etiology derived from traditional B-2 cells or a heterogeneous etiology varying from patient to patient [1, 16, 17]. While we cannot yet conclude the originating cell type of CLL or how the CLL lineage may play a role in the heterogeneity of the CLL disease course, evidence suggests a similarity between CLL cells and the B-1 cell phenotype. Data in support of this

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hypothesis will be summarized in the remainder of this short review.

## SIGNALING MOLECULES: SENSITIVITY AND EXPRESSION

The B-1 subset of lymphocytes represents a unique B-cell profile. This profile includes an autocrine signaling pathway through interleukin 10 (IL-10), increased surface expression of CXCR5 as compared to B-2 cells, and potential survival and proliferation through the soluble protein APRIL (a proliferation-inducing ligand) [18-20]. Interestingly, CLL cells have been shown to utilize similar signaling mechanisms for their own growth and survival (Fig. **1**). For example, serum levels of IL-10 are significantly higher in CLL patients than control subjects, and elevated levels correlated with poor overall survival [21]. A murine model of CLL with malignant B-1 cells displayed higher IL-10 mRNA levels than normal B-2 or B-1 cells [12]. Additionally, recent *in vitro* data suggest that CLL cells cultured *ex vivo* show a marked decrease in IL-10 levels, which also corresponds with the induction of spontaneous apoptosis in the cells [22]. Under physiologic conditions, IL-10 is primarily produced by B-1 cells as compared to other B cell types. This cytokine, which is a known regulator of humoral response activation, is also thought to be an important growth factor for B-1 cells, allowing for their unique longevity and auto-renewing properties [18, 23]. Interestingly, the CD5 surface marker that is found on B-1 and CLL cells may be facilitating the upregulation of IL-10 that leads to autocrine pro-survival signaling in these cells [24] thus highlighting the similar functions IL-10 appears to perform in both the B-1 and CLL cells.

In fact, IL-10 is not the only cytokine with similar production and effects in both B-1 and CLL cells. Similar to IL-10 serum levels in CLL patients, levels of the pyrogenic cytokine interleukin-6 (IL-6) in CLL serum also appear to be elevated and correspond to an aggressive disease state [21]. Not only have B-1 cells been characterized as producers of IL-6, but Cong *et al*. (1991) reported that a combination of IL-6 and anti-IgM in culture can induce CD5 expression in previously CD5-negative murine B cells and confer a B-1 phenotype [25, 26]. Therefore, IL-6 may be involved in the promotion and/or maintenance of CLL and B-1 cells.

Another cytokine that appears to function in both B-1 and CLL cells is interleukin-5 (IL-5). It has been shown that the IL-5 receptor is expressed by CD5+ B-1 cells, which secrete IgM upon stimulation with IL-5 [27]. As it is known that many B-1 antibodies are auto-reactive, an over-production of IL-5 leading to expansion of B-1 cells should increase the susceptibility to auto-immune disease. However, a study performed in an IL-5 transgenic mouse susceptible to systemic lupus erythematosus (SLE) showed that the increase in B-1 cells was protective against SLE while causing a CLL phenotype in the mice [28]. This study therefore highlights the importance of IL-5 in the functioning of B-1 cells and suggests a potential link between B-1 and CLL cells, at least in this murine model.

The chemokine receptor-ligand interaction between CXCR5 and CXCL13 has recently been studied for its role in the homing, proliferation, and survival of CLL cells. Data suggest that overexpression of CXCR5 by CLL cells allows them to home to lymphoid tissues where they can interact with CD68+ stromal cells that provide the CLL cells with important pro-survival signals. Additionally, CLL patients exhibit significantly higher levels of CXCL13 in serum than control subjects, suggesting a role for this CXCR5-CXCL13 interaction in the disease state [29]. Similar to CLL cells, B-1 cells have been shown to express higher levels of CXCR5 than their B-2 cell counterparts, and this expression is necessary for the B-1 cells to home to the peritoneal cavities where they function in immune response *via* antibody production [19].

Yet another molecule that may play a role in the proliferation/survival of both CLL and B-1 cells is APRIL. This soluble protein has been shown to activate the NFκB pathway in CLL cells, leading to resistance to apoptosis and prolonged survival. APRIL has also been found to be elevated in CLL patients as compared to control subjects [20, 30]. Interestingly, overexpression of APRIL in transgenic mice confers an abnormal expansion of B-1 cells in the peritoneal cavities that leads to lymphoid tumor development in this murine model [20]. Thus, these studies suggest a tumorigenic role for APRIL in B-1 cells and CLL.

NF-AT (nuclear factor of activated T cells) is another signaling molecule that has been implicated in the activation of both CLL and B-1 cells. In B-2 cells, NF-AT appears to be activated upon BCR crosslinking, whereas its expression is constitutive in B-1 cells [15]. A similar phenomenon is seen in CLL cells, where a patient's normal B cells can be distinguished from transformed CLL cells by the constitutive nuclear localization of NF-AT in the CLL cells [31]. The increased expression of NF-AT in CLL samples has also been directly correlated with increased CD38 expression, which is considered an indicator of poor clinical prognosis [32]. In addition, the activation of NF-AT may be of particular importance to B-1 and CLL cells, as studies have shown that NF-AT has multiple binding sites for the CD5 gene and may therefore regulate the characteristic expression of CD5 in these cell types [15, 33]. Taken together, these data indicate similar signaling patterns may be responsible for the regulation of both B-1 and CLL cells, highlighting the cells' resemblance.

## BCR SPECIFICITY

One of the unique characteristics of B-1 cells is their BCR specificities. The B-1 cells are thought to be a link between innate and adaptive immunity because they have BCRs with natural- and auto-reactivity [34]. These BCRs include a limited repertoire of specificities with heavy- and light-chain selectivity in the B-1-cell subsets that are thought to play a pivotal role in T-independent/innate immune responses [8, 35]. Interestingly, many CLL cases have been shown to have similar BCR characteristics as B-1 cells. At least 20% of CLL patients have been characterized as having stereotypic heavy chain complementarity-determining region 3 sequences [36]. In particular, the BCRs of CLL patients tend to exhibit outgrowth of the same immunoglobulin clones that utilize limited Ig genes such as $V_H5$, $V_H4$, $V_KI$, and the G6 cross-reactive idiotype [37-39]. Also, many antibodies produced from CLL cells are able to bind apoptotic cells, recognizing self-antigens associated with apoptosis and/or cellular stress. These antibodies also tend to

be unmutated and auto- and poly-reactive — a phenotype similar to the B-1 cells [13, 16].

## IMMUNO-SUPPRESSIVE PROFILE: B REGULATORY LINK?

Although a role for the T regulatory cell has been substantiated within the immune repertoire for some time, it is only in the past few years that researchers have appreciated a humoral component for immune down-modulation: the $CD5^+CD1d^+CD11b^+$ B regulatory cell (Breg). While not much is known about this subset of cells, Bregs are thought to cause immune-suppression through complex interactions with other immune effector and stromal cells mediated by the Bregs' production of IL-10 [40, 41]. Certain IL-10-secreting Bregs are expanded in cases of endogenous autoimmunity, whereas cases of exogenously-perturbed autoimmune disorders have corresponded with decreased numbers of Breg cells. This suggests a complex paradoxical relationship between immune regulation and auto-immunity [42]. Interestingly, this same contradictory relationship has also been seen with B-1 cells, where B-1 cells have been associated with the generation of immune tolerance and with the induction of autoimmunity [26, 35].

In addition to being the primary B cell producers of IL-10, further evidence suggests that B-1 cells may compose at least a proportion of the recently discovered Bregs [26, 43]. In support of this hypothesis, B-1 cells have been previously suggested to play an important role in immune tolerance and down-modulation [15, 26, 35]. Recent data from our laboratory also suggests an immune-suppressive or tolerogenic signature in many CLL patient cases based on microarray analysis. Our results indicate that microenvironmental interactions allow CLL cells to elicit tolerizing signals to surrounding immune cells [44]. In contrast to producing an immunosuppressive environment, autoimmune disorders often co-present with CLL, suggesting that CLL cells may also be involved in complex immune-regulatory processes similar to B-1 and Breg cells. These processes could potentially be mediated through increased IL-10 expression and other immune-modulatory interactions in various microenvironmental niches [21, 45]. These evidences suggest that there may be an etiological link between B-1, Breg, and CLL cells (Fig. **1**), as their phenotypic (such as CD5, CD23, and CD43 expression) and discussed functional aspects bear strong resemblance to each other in multiple contexts.

## ONGOING STUDIES

While there are substantial similarities between B-1 and CLL cells, some researchers still question the etiological origin of CLL and suggest that B-1 cells may not necessarily be responsible for CLL leukemogenesis. For example, some argue that the CD5 marker present on both B-1 and CLL cells may not be an indication of common origin, but rather a marker for a specific differentiation or activation state in the cells [1, 46]. This idea is supported by evidence that incubation with IL-6 and anti-IgM can induce CD5 expression and confer a B-1 cell phenotype in CD5-negative murine B-1 cells [25]. Others suggest that there may not be one single etiological origin for CLL and that the heterogeneity of clinical disease may actually derive from different CLL cases having diverse pre-cancerous origins. For example, a recent study identified two groups of CLL cases: one group having heterogeneous BCRs and the other having stereotypical BCRs. The authors suggested this result may be attributed to certain CLL cases arising from traditional B-2 cells (those with heterogeneous BCRs), while other CLL cases may arise from B-1 cells (those having stereotypical BCRs) [17]. Therefore, while evidence exists in support of the hypothesis that CLL cells are derived from B-1 cells, this data is not conclusive, leaving the etiological origin of CLL cells an unsolved mystery.

Given this information, future studies are necessary to resolve the predecessor of the CLL cell. Gratuitously, current methodologies and models lend themselves to this task. For example, despite the lack of a characteristic translocation associated with leukemogenesis in CLL, it is possible that other alterations or epigenetic changes may be taking place. The varying roles of methylation, histone modification, and miRNAs in promoting the leukemic phenotype have all been recently reviewed [47]. Therefore, the epigenetic patterns of B-1, B-2, and CLL cells may not only provide insight as to the etiology of CLL, but may also help determine the confounding factors leading to cancer initiation in CLL patients.

In addition to looking at the epigenetics of CLL, it may also be pertinent to further standardize and utilize murine models that recapitulate the CLL phenotype. In fact, a recent animal model of CLL, the Eμ-TCL1 transgenic mouse, has been utilized to investigate epigenetic changes in CLL that are correlative to patient cases, thus highlighting its value in investigating the CLL phenotype [48, 49]. Also, as CLL cells are known to readily undergo apoptosis *in vitro*, microenvironmental and immune-modulatory studies are vital to understanding the role of the various niches of CLL and how they promote survival and proliferation; these studies may only be possible through utilization of an animal model.

## CONCLUSION

In conclusion, the etiology of CLL is still not entirely understood. The unique phenotype of these cells expressing both B and T cell molecules, various different activation markers, and unique signaling molecules all confound our understanding of their original derivation. However, evidence does suggest based on their signaling molecules expression and sensitivity, BCR specificity, and unique immune-modulatory profile that these cells share a similar phenotype with the B-1 cells of the immune system (Fig. **1**). Through further investigation of the normal function of B-1 cells as well as their potential actions as Bregs, we may be able to determine the instigating factor—whether it be through a pathogenic insult or a combination of genomic and epigenetic events—leading to leukemogenesis in CLL patients. Additionally, investigating the roles of B-1 and Bregs in their normal physiological niches may facilitate our understanding of the unique mechanisms by which CLL cells exhibit prolonged survival, immune modulation, and other effects that play a role in the tumorigenesis of this heterogeneous disease.

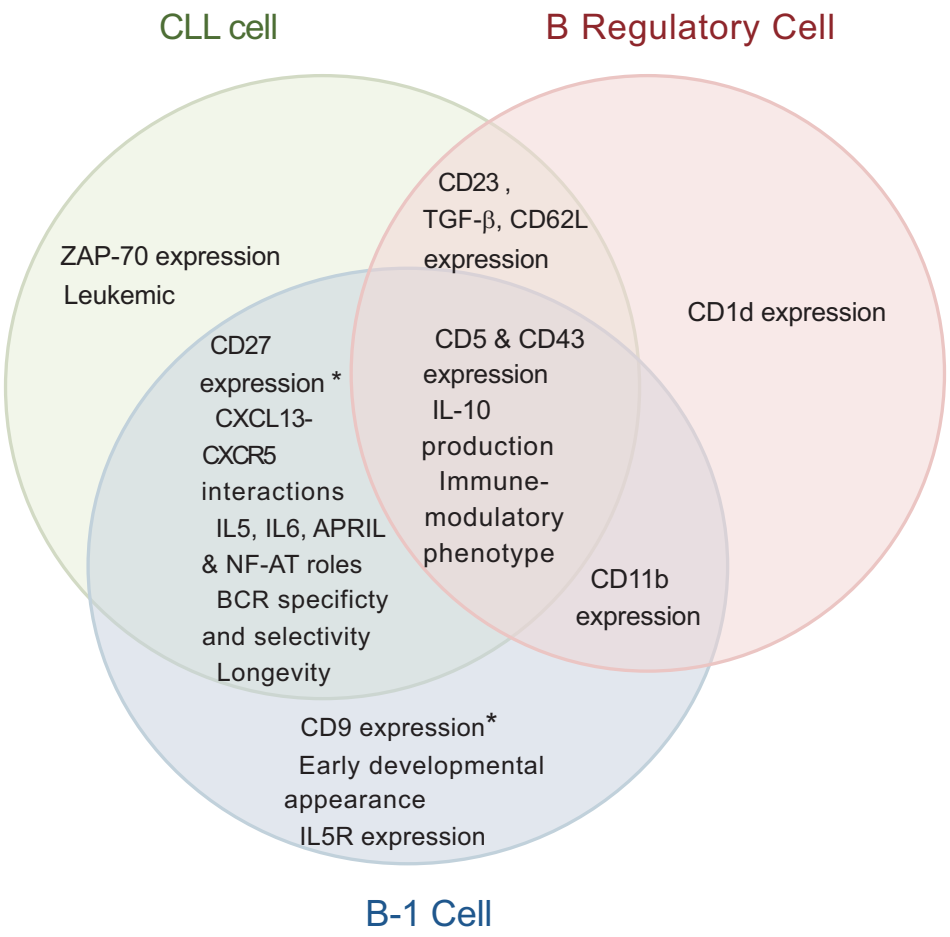


**Fig. (1).** Representative Venn diagram highlighting numerous similarities and differences in expression and/or phenotypic patterns between CLL, B-1, and B Regulatory cells, as suggested by current literature. * *Denotes notable similarities also shared with MZ B cells* [16, 19, 20, 26, 29, 30, 34, 50-53].

## CONFLICT OF INTEREST

The authors of this manuscript have no conflicts of interest in publishing this review, and do not have any financial relationship with commercial entities with interest in CLL.

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