Case Report

Spontaneous Regression Accompanied by Concomitant Immune Alterations in a Patient with Chronic Lymphocytic Leukemia

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Abstract

Spontaneous regression (SR) of chronic lymphocytic leukemia (CLL) is a rare event (0.2% - 1%). Some advances have been made in understanding the tumor genetic characteristics of such patients, although the immunological mechanisms leading to SR remain unclear. We describe a series of immunological events related to regression dynamics, allowing the identification of a SR phase (associated with >99% reduction of CLL cells in peripheral blood and adenopathy resolution in less than one year, concurrently with a nine-fold increase in monocyte counts, high B2M and the appearance of an oligoclonal serum IgG band), followed by a persistent regression (PR) phase that was maintained for ≥17 months. Our observations highlight a role of monocytes and B2M in SR, potentially related to immune activation. The oligoclonal IgG band detected during SR was maintained in PR, suggesting either a change in the ability of malignant cells (IgM⁺IgD⁺IgG⁻) to differentiate into IgG-secreting cells, or an anti-tumor humoral response from normal B cells. These findings imply immune and molecular mechanisms required to eliminate malignant cells and might suggest new immunotherapies for CLL.

Introduction

Chronic lymphocytic leukemia (CLL) has a heterogenous clinical course, ranging from indolent to rapid progression. Spontaneous regression (SR) is a rare event [1-4]. Nakhla and colleagues reported a frequency of 0.6% [3], similar to the 0.2%-1% recently reported by the European Research Initiative on CLL (ERIC) registry [1]. Accepted criteria for CLL regression are a diagnosis of CLL consistent with iwCLL guidelines [5] followed by a lymphocyte count decrease of ≥50% of the highest value, normalization of clonal B-cell numbers to <5x10⁹/L, and resolution

of other features such as lymphadenopathy or splenomegaly that continue for >12 months, all in the absence of intervening treatment [1].

In CLL, a broad range of alterations in patient T cells occurs [6-8], presumably originating from persistent exposure to malignant cells. In addition to T cells, myeloid cell subsets found in CLL patients differ from those of healthy individuals. Augmented numbers of monocytes that exhibit dysregulated phagocytosis and inflammatory capabilities exist in the peripheral blood (PB) of CLL patients [9], as well as greater numbers of myeloid-derived suppressor cells, the latter promoting tumor progression [10]. In advanced stages of the disease, the expansion of clonal B cells goes together with significant inhibition of the immune system [11], thereby diminishing patients' capacity to mount an effective antitumor defense. In summary, besides the biological characteristics of the malignant clone, an increasing amount of evidence highlights the importance of the surrounding immune environment in tumor expansion, tumor control or, at the other end of the spectrum, SR [2].

On certain occasions, tumor regression associates with external factors such as infections, blood transfusions, or the emergence of secondary malignancies, potentially reflecting the induction of a proinflammatory immune environment [12, 13]. In this context, we recently described an increased inflammatory signature in patients with the pre-leukemic stage of CLL, referred to as monoclonal B-cell lymphocytosis (MBL) [14]. Both CLL SR and MBL could be viewed as scenarios in which the proliferation of CLL cells is somehow controlled or even reduced. As a result, it could be hypothesized that there are common immune mechanisms at play in people who underwent CLL SR and patients with MBL. A comprehensive understanding of the immune mechanisms underlying these favorable disease scenarios will help

decipher CLL's intricate pathobiology and guide to develop novel therapeutic strategies with a focus on disease prevention.

Case Report

During a routine health exam in April 2020, a 69-year-old male, who was otherwise healthy without recurrent infections, reported the appearance of enlarged supraclavicular and cervical lymph nodes. After clinical, hematological, and immunological assessments, a clonal B-cell population >5x10^9 cells/L with a CLL-specific immunophenotype (CD5$^+$, CD19$^+$, CD23$^+$, CD20^{low}, CD10$^-$, CD43$^+$, CD79b$^-$, CD200$^+$, sIgκ^{low}) was documented, and the patient was diagnosed with CLL, Rai stage I the following month. Computerized tomography showed widespread lymphadenopathy bilaterally in the cervical and thoracic regions and smaller nodes in the abdomen and pelvis. Spleen and liver sizes were normal.

The patient's hematological profile changed over a 4.5-year period (Figure 1A). Circulating lymphocytes doubled from 4.8x10^9 cells/L in October 2018 to 9.3x10^9/L in April 2020 (reference values: 0.8-4.0). In May 2020, the B-cell clone comprised 61% of circulating lymphocytes (Table 1), corresponding to 5.2x10^9 cells/L. However, in February 2021, the patient's total lymphocyte count was documented within reference values (2.5x10^9/L), where it has remained for nearly two years (range: 1.9-3.0x10^9/L, Figure 1A). In May 2021, clonal B cells reached its minimum value (2% of lymphocytes, corresponding to 0.04x10^9 cells/L, which was a >99% reduction of the B-cell clone), although complete regression was not achieved until August 2021, when supraclavicular lymph nodes were no longer palpable. Considering all these parameters, SR began in or after August 2020 (last record of high lymphocyte counts) and was completely achieved in August 2021, following which a persistent regression (PR) was maintained for at least 17 months (Figures 1A and 1B).

Parameter	CLL	Spontaneous regression		Persistent regression			
	May-20	Mar-21	May-21	Sep-21	Jan-22	Jul-22	Jan-23
Lymphocytes (% total white cells)	50	32	29	21	23	19	24
T cells (% lymphocytes)	25	53	61	59	63	51	45
CD4:CD8	1.8	1.8	1.4	1.9	2.1	2.2	1.9
NK cells (% lymphocytes)	6	18	31	25	15	22	17
Total B cells (% lymphocytes)	62	22	3	11	17	21	35
Clonal B cells (% total B cells)	98	95	67	55	65	67	83
Normal B cells (% total B cells)	2	5	33	45	35	33	17
Clonal B cells (% lymphocytes)	61	21	2	6	11	14	29
Kappa:lambda (immunoassay)	97.6	48.4	4.2	3.5	4.6	6.4	14.8

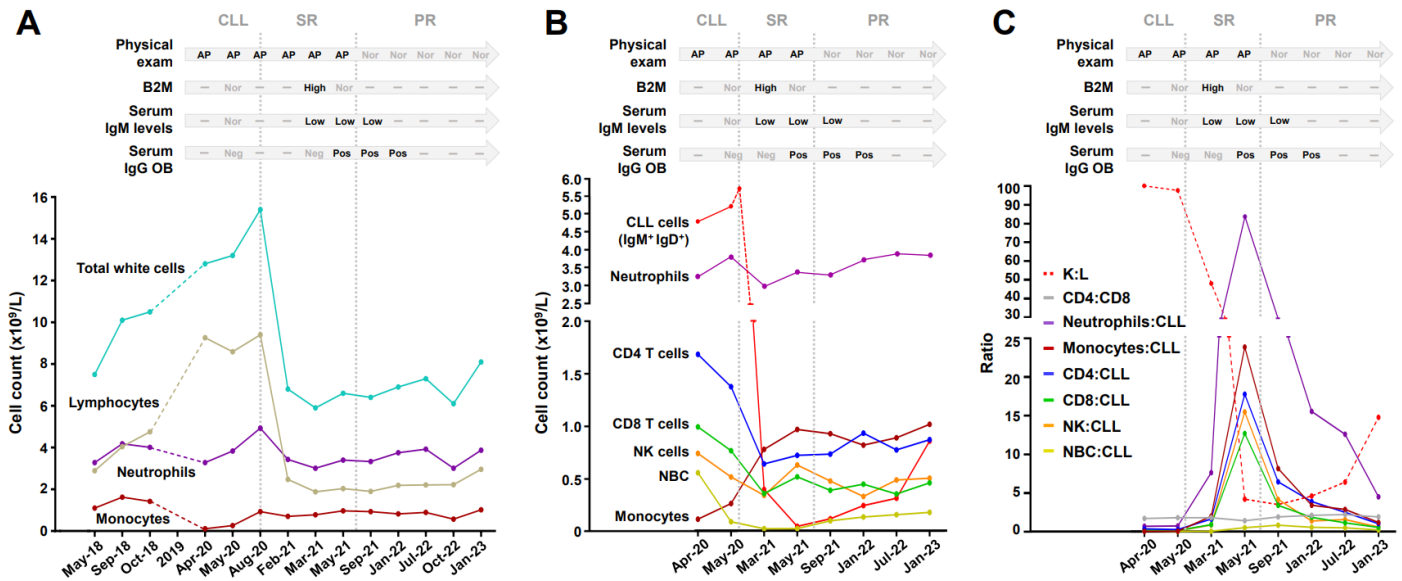**Table 1:** Flow cytometry analysis of peripheral blood.

Figure 1: Evolution of the hematological profile of a patient diagnosed with CLL who underwent spontaneous regression. Physical examination, serum B2M and IgM levels, and results of serum immunofixation are detailed. A) Changes in total white cells, total lymphocytes, neutrophils, and monocytes over a 4.5-year period. Data were not available between October 2018 and April 2020, as represented by dotted lines. B) Changes in absolute numbers of specific immune cell subsets after CLL diagnosis. C) Immune cells to clonal B-cell ratios, CD4:CD8 T-cell ratios, and kappa:lambda ratios measured by immunoassay. SR, spontaneous regression; PR, persistent regression; AP, adenopathy; Nor, normal; B2M, β-2 microglobulin; OB, oligoclonal band; Neg, negative (normal polyclonal pattern); Pos, positive (IgG oligoclonal band); NBC, normal B cells; K:L, kappa:lambda light chain ratio.

Notably, starting at the initiation of SR, absolute numbers of T and NK cells decreased, although their percentages among total lymphocytes increased (Figure 1B, Table 1). Numbers of neutrophils were more stable over time and always within normal values ($3.0\text{-}4.9\text{x}10^9$/L, reference values: 1.5-6.5). Most striking, however, was the increase in monocyte numbers detected concurrently with tumor regression, from $0.11\text{x}10^9$/L in April 2020 to 0.97 in May 2021, the latter being higher than reference values (0-0.9). Monocyte counts were maintained at the upper limits of normal during PR (Figure 1B). Moreover, levels of β-2 microglobulin (B2M), which has prognostic relevance in CLL [15], were normal at diagnosis, increased into the abnormal range (>3 g/L) at the beginning of SR (when most tumor cells were eliminated), and then returned to normal (Figure 1B, Table 2). Although plasma IgG and IgA were within normal levels, IgM decreased during SR and was maintained below reference values during PR, until the last measurement; this correlated with the fall in CLL cells expressing IgM (Figure 2, Table 2). Serum protein immunofixation exhibited a polyclonal pattern at CLL diagnosis and at the beginning of SR. However, since May 2021, concurrent with a progressive slow increase of normal and clonal B cells, an oligoclonal band of the IgG isotype appeared and was maintained from the end of SR until the last measurement in PR (Figure 1B, Table 2). To determine if this oligoclonal IgG was produced by malignant cells, a flow cytometry isotype study was performed. Clonal B cells expressed IgM and IgD, but not IgG (Figure 2). Throughout the patient's clinical course, total protein and albumin levels were normal, as well as those for hemoglobin, platelets, and C-reactive protein.

Protein	CLL	Spontaneous regression		Persistent regression		Reference values*
	May-20	Mar-21	May-21	Sep-21	Jan-22	
IgG (g/L)	10.7	9.94	12.6	11.5	-	5.76-15.36
IgA (g/L)	1.96	1.85	1.9	1.87	-	1.24-4.16
IgM (g/L)	0.48	0.38 (low)	0.44 (low)	0.44 (low)	-	0.48-3.1
Free Kappa (N-latex)# (mg/L)	35 (high)	38 (high)	30 (high)	30 (high)	36 (high)	7-22
Free Lambda (N-latex)# (mg/L)	21	20	17	21	19	8-27
Free K:L ratio (N-latex)#	1.67 (high)	1.90 (high)	1.76 (high)	1.43	1.89 (high)	0.31-1.56
Immunofixation	Negative	Negative	OB	OB	OB	
B2M (g/L)	2.28	3.14 (high)	2.62	-	-	<3
*Sonic Health Reference Intervals. #Latex-enhanced monoclonal antibody assays that measure free light chains. Ig, immunoglobulin; OB, oligoclonal bands (IgG, Igκ and Igλ oligoclonal bands); B2M, beta 2 microglobulin.						

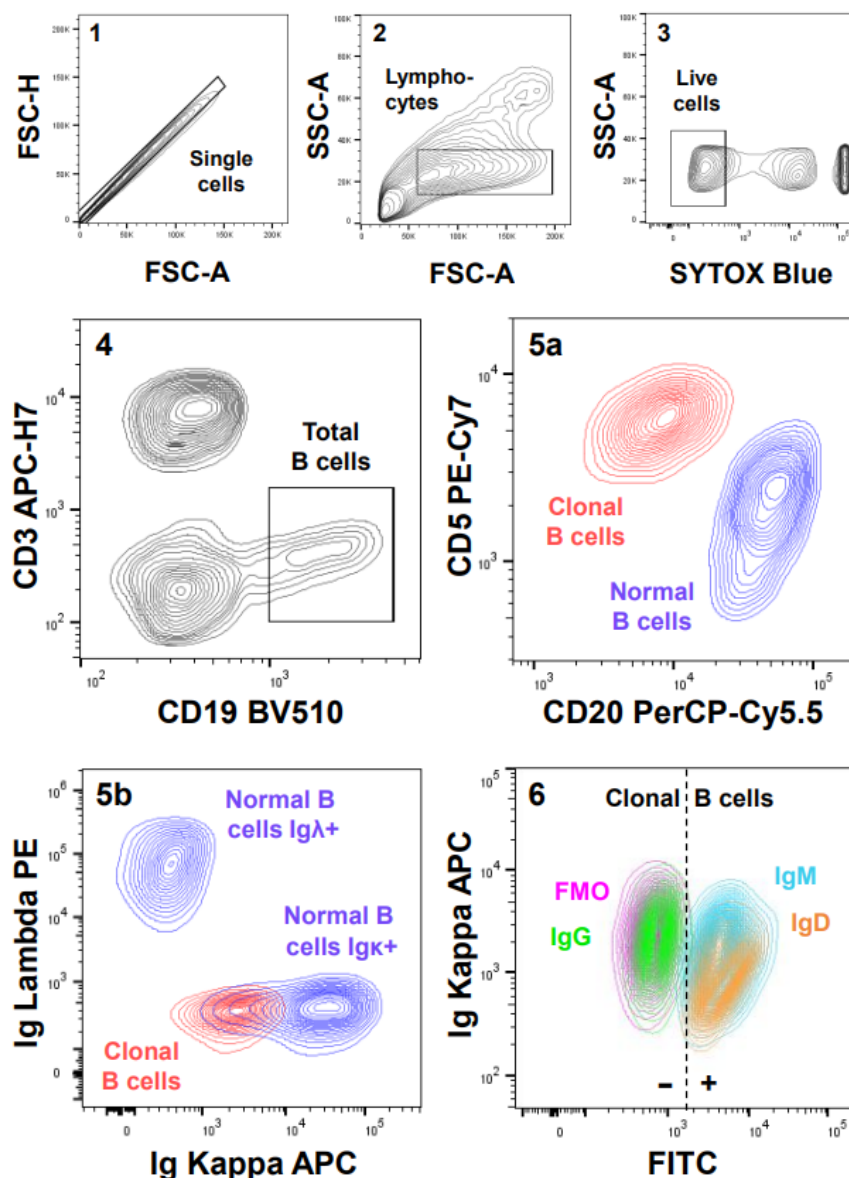**Table 2:** Monoclonal assays of plasma proteins.

Figure 2: Flow cytometry gating strategy for the immunoglobulin (Ig) heavy chain isotype analysis of clonal B cells. After gating for single (1) lymphocytes (2), SYTOX Blue was used to select viable cells (3). CD3⁻ CD19⁺ cells (total B cells) were gated (4) and clonal B cells were discriminated from normal B cells based on CD5⁺ CD20^low expression (5a) and Igκ^low restriction (5b). Clonal B cells were IgM⁺ IgD⁺ but IgG⁻, whose expressions were evaluated employing a fluorescence-minus-one (FMO) control (6). In the contour plots, fluorescence intensity values were transformed into logarithmic scale.

Discussion

In the largest cohort studied so far, SR was observed after a median time of 8 years from diagnosis [1], which is in contrast with our case. Although our patient met international criteria for the diagnosis of CLL ($\geq 5 \times 10^9$ clonal B cells/L for at least 3 months [5]), the CLL phase was brief, between 3 and <10 months. This highlights the uniqueness of our patient, with an immune system potentially not severely impaired by malignant cells. In other studies, SR was associated with reversal of T-cell exhaustion and increased T-cell proliferation [2]. However, in our patient, absolute numbers of all lymphocyte types decreased at the beginning of SR, although numbers of the cytotoxic NK and CD8$^+$ T cell subsets slightly increased before the achievement of PR (Figure 1B). We and others have described tumor-related alterations of NK and T cells in CLL and in its pre-malignant stage, MBL, and antitumor effects exerted by these cell subsets have been reported [16-19]. The latter might have occurred in our case.

The frequent evaluations of our patient allowed the analysis of regression dynamics, distinguishing between a SR phase (>5 months to 1 year) and a PR phase of at least 17 months. Cell ratios between immune and clonal B cells showed clear peaks in May 2021, during SR (Figure 1C), consistent with a pronounced decrease in clonal B-cell counts. These peaks may also reflect an active function of immune cells in establishing a PR. Although high B2M is associated with an aggressive disease in CLL [15], B2M levels in our patient were elevated at the beginning of SR, after which time they normalized. Surprisingly, B2M gene expression was significantly higher in tumor cells of SR compared to those of stable and progressive CLL patients [4], consistent with this HLA-I component being required to allow tumor recognition and elimination by immune cells. Our case seems to confirm the previous findings and supports high B2M as a characteristic feature of SR, which may be related to anti-tumor immune eradication.

An oligoclonal serum IgG band appeared at the end of SR and was maintained during PR. The relevance of this to the clinical course of our patient is unclear, although two possibilities can be considered. First, this might represent a change in the ability of malignant cells (IgM$^+$IgD$^+$IgG$^-$) to differentiate into IgG-secreting cells. Previous studies demonstrated that terminal differentiation and IgG isotype switching can be accomplished by IgM$^+$ CLL cells [20]. However, not finding IgG in the clonal population mediates against this possibility (Figure 2). Second, the oligoclonal IgG band could be produced by normal B cells as part of an anti-tumor humoral response. Secreted IgG could specifically bind tumor cells and then be recognized by effector cells expressing Fc-gamma receptors (FcγR), such as CD16$^+$ NK cells or monocytes, to perform antibody-dependent cellular cytotoxicity (ADCC). In mice, non-classical monocytes (Ly6C^{low}) express all FcγRs required for IgG activity and are crucial for therapeutic IgG

efficacy [21]. In humans, non-classical monocytes expressing CD16 are, together with NK cells, essential for ADCC and can kill primary leukemic cells, and CD16$^+$ monocytes from leukemia patients can perform potent ADCC [22]. A recent study of 70 CLL patients indicated that percentages of non-classical CD16$^+$ monocytes decrease with the stage of CLL and are increased in patients bearing good prognostic markers, whereas those patients with reduced percentages are more likely to require treatment, indicating beneficial effects of CD16$^+$ non-classical monocytes on antitumor responses [23].

Additionally, associated clinical problems have been reported at SR in 46% of cases, mostly infections or second neoplasias [1]. Although none of these was present in our patient, the possibility of subclinical infections boosting immune cells cannot be formally excluded, and such an event could increase monocyte counts and, concurrently, trigger SR. This potential association, together with the increased monocyte inflammatory signature we previously described in MBL [14], highlights the importance of monocytes in tumor control, especially in those cases where malignant cells have not been able to profoundly impair the accompanying immune cells due to short disease duration and/or reduced tumor population. In detail, we previously described an increased inflammatory drive orchestrated mainly by monocytes in MBL patients compared to age-matched healthy controls. This inflammatory signature of monocytes was decreased in CLL patients compared with MBL [14], suggesting a potential association between monocyte function and the maintenance of a reduced clonal B cell population as that observed in MBL. In the case of the CLL patient undergoing SR reported here, monocytes were the main cell type for which there was an absolute increase in cell counts at the time of the decrease in clonal B cell counts. This also points to a role for monocytes in restraining malignant expansions. In this sense, Kwok and colleagues pointed to MBL as a similar condition where the low-risk genetic characteristics of pre-tumor cells are not sufficient to avoid malignant expansions, proposing an active role of the microenvironment both in MBL and SR [2].

Conclusion

This study describes for the first time a series of events (increase of monocytes and B2M concurrently with a decrease in serum IgM and the appearance of an oligoclonal serum IgG band) in a case with SR. These findings might shed light on the immune and molecular mechanisms required to eliminate malignant cells and might suggest new immunotherapies for CLL.

The presence of the oligoclonal IgG band observed in SR and maintained during PR suggests a shift in the capability of malignant B cells (IgM$^+$ IgD$^+$ IgG$^-$) to differentiate into IgG-secreting cells, or implies the activation of an anti-tumor humoral

response by normal B cells, accompanied by the other cellular orchestrators of ADCC, such as monocytes or $CD16^+$ NK cells. Additional investigations are required to completely address these questions.

Ethical Considerations: Informed consent was obtained from the patient involved in this study.

Conflict of Interest: The authors have no conflicts of interest to disclose.

Data Availability Statement: Data supporting this study are included within the manuscript. Other specific data are available on request.

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