**Case Report**

**CD20dim-positive T-cell large granular lymphocytic leukemia in a patient with concurrent hairy cell leukemia and plasma cell myeloma**

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**Abstract:** We report a CD20dim-positive T-cell large granular lymphocytic (T-LGL) leukemia in a patient with concurrent hairy cell leukemia and plasma cell myeloma. This patient was first diagnosed with T-LGL leukemia with dim CD20 expression, which by itself was a rare entity. He received no treatment for T-LGL leukemia. The patient later developed a hairy cell leukemia, which went into complete clinical remission after one cycle of 2-CdA. Five years later, he was diagnosed with a third malignancy, plasma cell myeloma. Complex cytogenetic aberrancies were present at the time when plasma cell myeloma was diagnosed. This is the first report, to the best of our knowledge, in the English literature with the aforementioned three distinct hematopoietic malignancies in one patient.

**Keywords:** CD20, T-cell large granular lymphocytic leukemia, hairy cell leukemia, plasma cell myeloma

**Introduction**

Large granular lymphocytic (LGL) leukemia is a rare form of leukemia of either cytotoxic T cells or natural killer (NK) cells. According to the current World Health Organization (WHO), T-cell LGL leukemia was defined as heterogeneous disorders with a persistent (more than 6 months in duration) increase of LGL in peripheral blood without a clearly identified cause [1]. Immuno-phenotypically, T-LGL leukemia could be divided into several variants with majority of the cases being CD3(+), CD4(-), CD8(+), CD57(+) and T-cell receptor (TCR) αβ[2]. Other minor variants included (1) CD3(+), TCRαβ(+), CD4(+), and CD8(-); (2) CD3(+), TCRαβ(+), CD4(+), and CD8(+); and (3) CD3(+) and TCRγδ(+). The CD4 and CD8 expression were not yet well defined in the latter variant [3].

CD20, a member of the membrane-spanning 4A gene family, encoded a cell surface protein. CD20 was expressed in late stage of hematogones but its expression was lost in the terminally differentiated plasma cells; therefore, CD20 was a mature B-cell associated antigen. Besides B-lymphocytes, dim CD20 expression was reported in a minute subset of T cells in normal individuals [4]. While there were estimated 23 cases of T-cell malignancies bearing CD20 expression [5], CD20-positive T-LGL leukemia was rare, and the present report is the fourth case of T-LGL leukemia with dim CD20 expression [5-7].

Hairy cell leukemia (HCL), first reported as leukemic reticuloendotheliosis [8], was a neoplasm of mature B cells characterized by an indolent clinical course with infiltration of peripheral blood, bone marrow, and red pulp of the spleen [9]. There were sporadic reports of secondary neoplasia associated with HCL, including acute myeloid leukemia, diffuse large B-cell lymphoma, peripheral T-cell lymphoma [10], monoclonal gammopathy of undermined significance [11], other myeloma-related neoplasms [12],
and chronic lymphocytic leukemia [13]. Besides the aforementioned population-based large studies [10-12] of surveying for secondary neoplasms in HCL, there were three case reports of HCL followed by plasma cell myeloma (PCM) [14-16], and one of the reports proposed a HCL transformation to PCM [15]. Also, HCL was reported in a case of monoclonal expansion of LGL cells with a CD4+/CD8dim+/- immunophenotype [17].

In the current study, we report a unique case of CD20 dim-positive T-LGL leukemia in a 69-year-old male who also has HCL and PCM. To the best of our knowledge, this is the first case in the English literature with three concurrent distinct hematopoietic neoplasms in a single patient.

Case report

A 69-year-old male was referred to the University of California San Diego Moores Cancer Center in 10/2004 for lymphocytosis, anemia, and thrombocytopenia. His complete blood count (CBC) in 10/2004 was as follows: WBC of 15.4 x 10^9/L (normal range: 4,000-11,000 x 10^9/L), RBC of 2.67 x 10^{12}/L (normal range: 4.7-5.7 x 10^{12}/L), hemoglobin of 8.8 gm/dL (normal range: 14-17 gm/dL), and PLT of 102 x 10^9/L (normal range: 130-400 x 10^9/L). Physical examination revealed a large spleen without lymphadenopathy. Based on the morphologic and immunophenotypic assessment of bone marrow (see below), the patient was diagnosed with T-LGL leukemia and lambda-restricted HCL. The cytogenetic analysis at that time revealed no abnormalities. The patient was then treated with one cycle of 2-CdA, and he had achieved a complete clinical remission with regard to his HCL. His symptoms were significantly improved with reduced splenomegaly, but his T-LGL leukemia persisted.

In early 2009, the patient again developed anemia and thrombocytopenia with a new onset of lambda-restricted para-protein. His CBC in 7/2009 was as follows: WBC of 13.2 x 10^9/L, RBC of 3.0 x 10^{12}/L, hemoglobin of 9.5 gm/dL, and PLT of 104 x 10^9/L. Besides persistent T-LGL leukemia mainly in the peripheral blood, a bone marrow biopsy revealed lambda-restricted PCM and residual HCL. The initial treatment for PCM was lenalidomide (Revlimid TM) plus dexamethasone (Decadron TM) in 8/2009. Due to the severe side effects, the treatment regimen was changed to bortezomib (Velcade TM)/doxorubicin (Doxil TM)/dexamethasone in 9/2009. He responded poorly to the chemotherapy with increased serum lambda immunoglobulin light chain, increased LDH, deteriorating renal function, and appearance of circulating plasma cells in the peripheral blood. He was admitted for salvage chemotherapy with DT-PACE regimen. On Day 40 of the salvage chemotherapy, besides his persistent T-LGL leukemia, a bone marrow biopsy revealed residual PCM and HCL. Unfortunately, the patient has since passed away and a post mortem examination has not been performed.

Materials and methods

Histomorphology

The peripheral blood and bone marrow aspirate smears were prepared and stained with Wright Giemsa according to standard procedures. The bone marrow core biopsies were first fixed in B5 (2 hours) and then 10% neutral formalin before decalcification. The bone marrow clot section was fixed with 10% neutral formalin only. Physical examination revealed a large spleen without lymphadenopathy. Based on the morphologic and immunophenotypic assessment of bone marrow (see below), the patient was diagnosed with T-LGL leukemia and lambda-restricted HCL. The cytogenetic analysis at that time revealed no abnormalities. The patient was then treated with one cycle of 2-CdA, and he had achieved a complete clinical remission with regard to his HCL. His symptoms were significantly improved with reduced splenomegaly, but his T-LGL leukemia persisted.

Immunophenotyping by multiparameter flow cytometry

Single cell suspension from bone marrow aspirate and peripheral blood after lysis of red blood cells was stained with the following antibodies: CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD25, CD38, CD56, CD57, CD103, CD138, cytoplasmic and surface kappa, cytoplasmic and surface lambda, TCRα/β, and TCRγ/δ. The cells were analyzed by FACSCaliber (Becton Dickinson, San Jose, CA). Data were acquired using CELLQUEST software (Becton Dickinson) and analyzed by a Paint-A-Gate software (Becton Dickinson).

Immunohistochemistry

Immunohistochemical stains on bone marrow core biopsy and/or clot section using the following antibodies CD3 (1:50, Lab Vision, Fremont, CA), CD4 (1:20, Biocare Medical, Concord, CA),
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CD8 (1:80, Biocare Medical), CD138 (1:60, abD Serotec, Taleigh, NC), DBA.44 (1:40, Dako, Dakocytomation, Carointeria, CA), kappa immunoglobulin light chain (1:500, Biocare Medical), and lambda light chain (1:35, Biocare Medical), was performed using Dako Autostainer with Envision(+) Detection Kit (Dako).

Conventional cytogenetics and fluorescence in situ hybridization (FISH)

Conventional karyotype on metaphase cells from bone marrow aspirate was performed using G-banding technique. FISH on interphase nuclei was performed from the CD138-positive plasma cells sorted using magnetic beads and unsorted bone marrow cells, and 100-200 cells were counted for each probe. Chronic lymphocytic leukemia associated probes including the D12Z3 for enumeration of chromosome 12, CCND1/IgH for t(11;14)(q13;q32), ATM at 11q22.3, D13S319 for 13q14.3, LAMP1 for 13q34, p53 at17p13.1, and myeloma associated probes including FGFR3/IgH for t(4;14), MAF/IgH for t(14;16), EGR1 for Sq31, D7S486 for 7q31 were analyzed by FISH. In addition, a FGFR1 (fibroblast growth factor receptor 1) FISH assay was also performed. All FISH probes were purchased from Vysis Inc. (Vysis Inc., Des Plaines, IL), except FGFR1, which was purchased from Veridex LLC (Raritan, NJ). Genetic abnormalities were classified according to the International System for Human Nomenclature [18].

Results

Morphologic findings

Concomitant peripheral blood smears from the 2nd and 3rd bone marrow biopsies (the slide from the first peripheral blood smear was lost) revealed 53-90% of total cells as atypical LGLs with oval to occasional irregular nuclear contours, some of which eccentrically located, coarse chromatin, absent nucleoli, and abundant pale basophilic cytoplasm and sparse azurophilic granules (Figure 1A). While not present in the peripheral blood smears from the 2nd and 3rd bone marrow specimens, the 1st peripheral blood smear was reported to have approximately 20% medium-sized atypical lymphoid cells with abundant cytoplasm exhibiting circumferential cytoplasmic projections, consistent with hairy cells (data not shown). In addition, there was an agranulocytosis and monocytopenia seen.

Due to lack of spicules, bone marrow aspirate smear from the 1st bone marrow specimen was not made. Bone marrow aspirate from the 2nd specimen showed occasional (less than 5%) atypical LGLs with similar morphologic features to those from the peripheral blood. However, the prominent feature of the bone marrow aspirate from the 2nd specimen was presence of numerous plasma cells (Figure 1B). These plasma cells accounted for approximately 80% of total cellularity based on 200-cell differential count.

Bone marrow core biopsy from the 1st specimen was approximately 80-90% cellular (Figure 1C), and approximately 80% of which were composed of small to medium sized lymphoid cells with irregular nuclear contours and abundant clear to pale cytoplasm with the so-called “fried egg” appearance (Figure 1D). However, no plasma cells were noted. Other normal hematolymphoid cells were present but in decreased numbers.

Bone marrow core biopsy from the 2nd specimen showed an uneven and patchy cellular distribution from less than 5% to focal 60% with an average cellularity of 30%. The cardinal feature was the interstitial plasma cell infiltrate forming sheets and small clusters. These plasma cells constitute ~70% of total cellularity (Figure 1E). Of note, atypical lymphoid cells were not easily noted. Bone marrow biopsy from the 3rd specimen showed a marrow of approximately 20% cellularity with scattered plasma cells.

Flow cytometry

Immunophenotypic analysis of the 1st bone marrow aspirate by flow cytometry revealed two distinct populations. The first population (yellow) represented 44% medium-sized cells which were positive for CD2 (Figure 2B), CD3 (Figure 2C), CD5 (Figure 2C & F), CD7 (Figure 2B), CD8 (Figure 2D), CD16/CD56 (Figure 2E), CD20 (dim) (Figure 2H), CD57 (variable) (data not shown), TCRα/β (not shown), but negative for CD1a (data not shown), CD4 (Figure 2D), CD19 (Figure 2F), CD23 (Figure 2H), and TCRγ/δ (data not shown), consistent with T-LGLs. The second population (red) represented ~20% medium-sized cells which were positive for CD19 (Figure...
Figure 1. Morphologic features of the T-LGL leukemia, HCL, and PCM in the peripheral blood and bone marrow. A. The predominant cells seen in the concurrent peripheral blood smear of the 2nd bone marrow biopsy were numerous medium-sized lymphoid cells with oval to irregular nuclear contours, homogeneously stained chromatin, abundant cytoplasm with sparse azurophilic granules, consistent with atypical large granular lymphocytes (Wright-Giemsa, original magnification 1000x); B. Numerous plasma cells with peri-nuclear hof were easily appreciated from the 2nd bone marrow aspirate (Wright-Giemsa, original magnification 500x); C. The hypercellular marrow from the 1st bone marrow biopsy was composed of predominantly small to medium sized lymphoid cells with an interstitial and nodular infiltrative pattern (H&E, original magnification 100x); D. The infiltrative lymphoid cells had abundant pale to clear cytoplasm, exhibiting the so-called “fried egg” appearance (H&E, original magnification 400x); E. The plasma cells from the 2nd bone marrow specimen formed sheets in the interstitium (H&E, original magnification 400x).
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Figure 2. (See figure legend next page)
CD20dim-positive T-LGL leukemia with hairy cell leukemia and plasma cell myeloma

Flow cytometric analysis of the T-LGL leukemia, HCL, and PCM on bone marrow aspirates. Compared to the small mature normal T-cells (population in green, A, D-E), the neoplastic T-lineage cells (population in yellow) were slightly larger in size (A), positive for CD2 (B), CD3 (C), CD5 (C), CD7 (B), CD8 (D), CD16/CD56 (E), and dim positive for CD20 (H), but negative for CD4 (D). The neoplastic B-lineage cells (population in red) were slightly larger in size (A), positive for CD19 (F & K), CD20 (H), dim positive for CD25 (I), CD103 (J), and surface lambda light chain restriction (K), but negative for CD5 (F), CD10 (G), and CD23 (H). The plasma cells (population in violet) were large-sized cells (A) with bright CD138 (L) and expressed cytoplasmic lambda light chain (L).

Immunohistochemical features

To confirm our suspicion that the 40% of aberrant LGL cells as revealed by flow cytometry on the 1st bone marrow aspirate was due to peripheral blood contamination, we resorted to immunohistochemical stains on the bone marrow core biopsy. As shown in Figure 3 A-C, there were less than 1% of CD3 (+) (Figure 3A), CD8 (+) (Figure 3B) but CD4 (-) (Figure 3C) T-cells in the marrow, indicating that the bone marrow was minimally, if any, involved by the patient’s T-LGL leukemia.

In order to corroborate the percentage of plasma cells obtained by differential count based on the bone marrow aspirate, a panel of immunohistochemistry was performed on the clot section from the 2nd bone marrow biopsy. CD138 staining (Figure 3D) showed that plasma cells accounted for ~70% of total cellularity. These plasma cells were positive for lambda (Figure 3E) but negative for kappa (Figure 3F) immunoglobulin light chain expression.

Conventional cytogenetics and FISH

Conventional cytogenetic and FISH analysis on cells from the aspirate of the 1st bone marrow specimen revealed no abnormalities. However, conventional karyotyping on cells from the aspirate of the 2nd bone marrow specimen (Figure 4) showed a complex abnormal karyotype as follows: 40-43,XY,-1,add(3)(p26), add(4)(p14),-6,psu dic(6;19)(q25;p13.3),der(8)t(8;17) (p21;q21),-10,add(11)(q23),-14,-17,-20,+mar [cp10] / 46,XY [25].

Conventional cytogenetic analysis of the bone marrow aspirate on the 3rd specimen showed a complex aberrant karyotype as follows: 40-41,XY,der(1)t(1;1)(?;?),?2q,-3, add(4)(p11.2),-6,del(7)(q31),der(8)t(8;17) (p11.2;q11.2),-10,add(11)(q23),add(14)(q32),del(17)(p12), add (19)(p13.3),-20,-20,+1-2mar[cp15]/46,XY [7].

Since breakpoint at 8p11.2 was seen on 2 consecutive bone marrow aspirates, a FGFR1 breakapart FISH analysis was performed on both CD138 sorted and unsorted cells. No 8p rearrangement involving the FGFR1 locus was observed among 200 cells analyzed.

Discussion

In the present study, we report an unusual case that harbored three distinct B- and T-cell lymphoproliferative disorders including CD20dim-positive T-LGL leukemia, HCL and PCM, the latter two of which shared identical immunoglobulin light chain expression. This case not only added one more case to the handful list of CD20-positive T-LGL leukemia, but also pro-
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Although CD20 is relative specific B-cell marker, aberrant CD20 expression has been reported in 16 cases of peripheral T-cell lymphoma [19], and dim CD20 expression has been observed in 3 cases of T-LGL leukemia [5-7]. While the precise mechanism of CD20 expression in T-lineage malignancy is not known, it has been postulated that the expression of CD20 in T-cell lymphoma/leukemia could be due to either aberrant acquisition of CD20 or clonal expansion of CD3 (+)/CD20 (dim +) T-cell subsets, the latter of which was favored by Quintanilla-Martinez et al [20], and supported by the findings that

Figure 3. Immunohistochemical features of the T-LGL leukemia and PCM in the bone marrow. A-C: Scattered CD3(+) (A), CD8(+) (B) and CD4(-) (C) T-cells were seen in the clot section (A-C: Original magnification 200x); D-F: The CD138 (+) (D) plasma cells were positive for lambda(+) (E) but negative for kappa (F) (D-F: original magnification 400x).
CD20 was expressed at a low level on a small subpopulation of T-cells [4], [20]. The expression of CD20 in T-cell lymphoma/leukemia not only bore diagnostic challenges and pitfalls [21], but also casted therapeutic significance, especially in the era of modern monoclonal antibody therapy. For example, Rituximab, an anti-CD20 monoclonal antibody, has been successfully used to eradicate the CD20 (+) peripheral T-cell lymphoma cells [22].

Our patient has 2 additional B-lineage neoplasms, namely HCL followed by PCM 5-years later, and both happened to be lambda immunoglobulin light chain restricted. While identical lambda light chain expression between HCL and PCM may raise the possibility of a clonal relationship between the HCL and PCM, the lack of CD19 expression among plasma cells (data not shown) in the PCM, on the other hand, may point to the opposite, since it has been shown that plasma cells derived from B-cell lymphoma usually retain CD19 expression [23]. Therefore, based on the data available, we were not certain of the clonal relationship between the HCL and the PCM in this case. Furthermore, molecular investigation of the ontogeny of PCM failed due to lack of suitable materials for a follow-up immunoglobulin heavy chain gene rearrangement study.

Both HCL and PCM were B-lineage neoplasms of post-germinal cell derivation, the former from late activated B-cells and the latter from long-
lived plasma cells. In a study of secondary cancer incidence from a cohort of 3104 patients with hairy cell leukemia with a mean follow-up 6.5 years [24], non-Hodgkin and Hodgkin lymphoma were significantly higher than the general population; however, increased incidence of PCM was not found [25]. This largest study to date raised the possibility that HCL and PCM, if occurring in the same subject, may just be coincidental rather than cause-effect. In fact, clonal relationship between HCL and PCM had not been established among 5 patients with both entities [14-16], [25-26]. While HCL was derived from post-germinal center activated memory B-cells, compared to other post-germinal center mature B-cell lymphomas such as marginal zone B-cell lymphoma and small lymphocytic lymphoma/chronic lymphocytic leukemia, HCL has not been reported so far to be associated with a related monotypic plasma cell population.

This patient had complex cytogenetic abnormalities including loss of multiple chromosomes, in particular, p53 locus on chromosome 17. Loss of p53 in PCM was associated with an inferior clinical outcome, which might explain the dismal clinical outcome in this patient [27]. Of note, this patient harbored an unbalanced translocation of 17q11.2 onto chromosome 8p11.2 to form a derivative chromosome 8, namely der(8)t (8;17)(p11.2;q11.2), in 15 out of 22 cells examined. Translocation of FGFR1 at 8p11 locus, a receptor tyrosine kinase, was typically involved in the so-called 8p11 myeloproliferative syndrome (EMS) /stem-cell leukemia-lymphoma syndrome [28]. To investigate whether FGFR1 at 8p11.2 was involved in this case, we performed FISH analysis using a FGFR1 breakapart probe, and the results showed no evidence for a FGFR1 rearrangement.

In summary, we report the first case of CD20 dim-positive T-LGL leukemia patient with concurrent lambda light chain restricted HCL and PCM. Due to lack of suitable specimen, the clonal relationship between the HCL and the PCM could not be established. The patient, unfortunately, succumbed to the PCM and passed away.

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