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*Case Report*

# Hairy Cell Leukemia with Unusual Loss of CD103 in a Subset of the Neoplastic Population: Immunophenotypic and Cell Cycle Analysis by Flow Cytometry

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**Abstract**: We report an unusual case of hairy cell leukemia (HCL) in a 55-year-old male who presented with fatigue, increased bruising, leukocytosis, anemia, thrombocytopenia and moderate splenomegaly without lymphadenopathy. Microscopically, a monomorphic population of small to medium-sized lymphoid cells with bean-shaped nuclei, ground glass chromatin and fine cytoplasmic projections was identified in the peripheral blood and bone marrow. Flow cytometric immunophenotyping demonstrated a monoclonal population of mature B cells with coexpression of CD25, CD11c and CD103. The clonal B-cells all exhibited homogenous expression of CD20 and uniform light scatter characteristics. However, CD103 expression was present in only half of the clonal B-cells. Flow cytometric cell cycle analysis using DRAQ5 DNA dye in intact live cells showed that both the CD103-positive and CD103-negative cell subsets exhibited a low S-phase fraction with no significant difference between the two subpopulations. Clinical remission was achieved by treatment with 2-chlorodeoxyadenosine. Variant and atypical cases of HCL have been described with varying intensity of CD11c, loss of CD25, aberrant expression of CD10, and lack of CD103 expression. However, the lack of CD103 in only a subset of the malignant cells in our case is an immunophenotypic aberrance that, to our knowledge, has not been previously reported.
**Key Words**: Hairy cell leukemia, flow cytometry, cell cycle, immunophenotype, DRAQ5, S-phase

## Introduction

Hairy cell leukemia (HCL) is a rare mature B-cell neoplasm, comprising 2% of lymphoid leukemias, with a predilection for middle-aged and elderly men. Patients commonly present with cytopenias, red pulp involvement of the spleen, and morphologically distinct, circulating cells with cytoplasmic projections and characteristic expression of CD25, CD11c and CD103 [1, 2]. This classic immunophenotype distinguishes HCL from other CD5-negative B-cell lymphoproliferative disorders, including the morphologically similar splenic marginal zone lymphoma with circulating villous lymphocytes [2]. Variant forms of HCL may present with a high white blood cell count or an altered immunophenotype and may respond suboptimally to certain types of chemotherapy [3, 4]. We report a case of HCL with a subset of the neoplastic population lacking CD103. We describe the morphologic and immunophenotypic features, including a novel, population-specific cell cycle phase evaluation and discuss the response to treatment and potential significance of our findings.

## Clinical History

A 55-year-old male initially presented with fatigue and increased bruising. The patient had no significant past medical history. A complete blood count revealed leukocytosis (20.1 x $10^3/mm^3$), anemia (hemoglobin 11.8 g/dL) and thrombocytopenia (platelet count 65 x $10^3/mm^3$). Results of a differential count included 96% lymphocytes with "cytoplasmic protrusions", 1% monocytes and 3% granulocytes. Radiological studies identified moderate splenomegaly without lymph-adenopathy.

Flow cytometric immunophenotyping of the

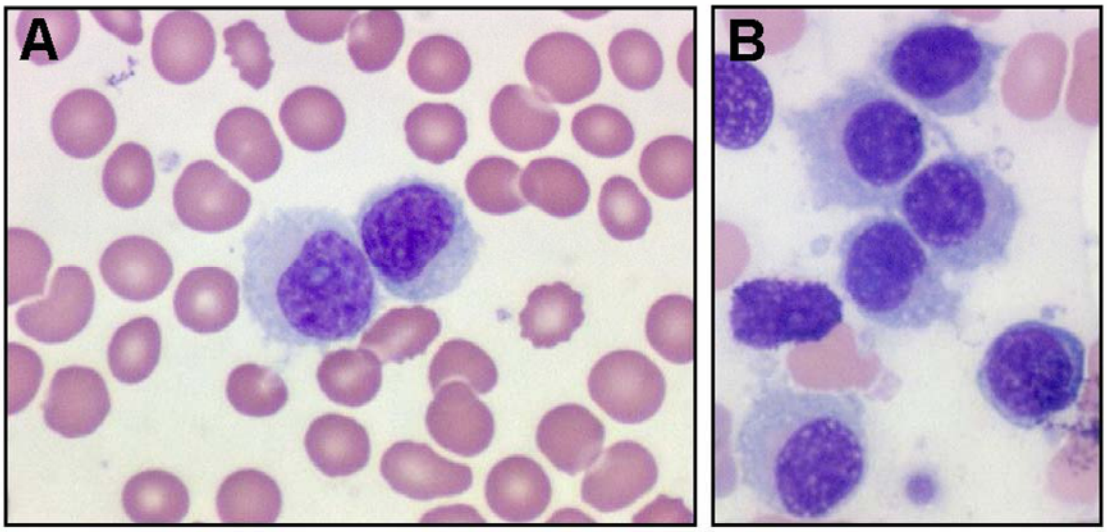


**Figure 1** Peripheral blood (**A**) and bone marrow aspirate (**B**) show the characteristic features of HCL cells (Wright stains, x1000).

peripheral blood showed a clonal population of mature B cells with kappa light chain restriction. The clonal B cells expressed CD25 and CD11c with an unusual bimodal CD103 expression. Microscopic evaluation of the peripheral blood identified a predominant, monomorphic population of small to medium-sized cells with fine cytoplasmic projections. The nuclei were bean-shaped, with spongy, ground-glass chromatin and occasional indistinct nucleoli that were morphologically consistent with HCL (**Figure 1A**). The result of a tartrate resistant acid phosphatase stain (TRAP) was equivocal. Morphologic and flow cytometric evaluation of a bone marrow aspirate showed similar findings with HCL involving 96% of the bone marrow aspirate (**Figure 1B**). The patient received 0.15mg/kg of 2–chlorodeoxyadenosine (2-CDA) each week for a total of 6 weeks. Three months later, the patient was re-evaluated and a bone marrow biopsy revealed adequate trilineage hematopoiesis and maturation with minimal residual disease. The aspirate contained <1% HCL cells. Other laboratory testing showed a normal complete blood count and electrolytes. CT scans showed resolution of splenomegaly. The patient exhibited a complete clinical response to treatment.

## Materials and Methods

*Sample Collection and Initial Preparation*

Bone marrow and peripheral blood samples were received in EDTA. Erythrocytes in the peripheral blood and bone marrow cell suspensions were lysed by incubating with 0.15M $NH_4Cl$ lysing solution for 10 minutes at room temperature at a ratio of 1:9 (volume of sample: volume of lysing solution), with a final volume of 50 mL. After incubation, cells were pelleted by centrifugation (500 g for 5 minutes at room temperature), the media was aspirated, and the cells washed twice in a phosphate-buffered saline solution containing 0.1%$NaN_3$ (PBS). After the final wash step, cells were resuspended in RPMI medium with 10% fetal calf serum containing a mixture of antibiotics.

*Cell Surface Staining*

Surface staining of the cells was performed in albumin (Sigma Chemical Company, Saint Louis, MO)-precoated wells in Falcon 96 well U-bottom assay plates (BD Labware, Franklin Lakes, NJ). The following antibodies were used: fluorescein isothiocyanate (FITC) conjugated kappa and lambda (Dako, Denmark) and CD103 (IQ Products, Netherlands) antibodies; phycoerythrin (PE) conjugated CD5, CD10, CD11c, CD23, CD25 antibodies (BD Biosciences, San Jose, CA); peridinin chlorophyll protein (Per-CP) conjugated CD45 antibody (BD Biosciences, San Jose, CA); allophycocyanin (APC) conjugated CD19 and CD20 antibodies (BD Biosciences, San Jose, CA). The antibodies were used in combination as part of a routine

panel as follows: kappa/CD5/CD45/CD19; kappa/CD10/CD45/CD20; lambda/CD11c /CD45/CD20; lambda/CD23/CD45/CD19; and CD103/CD25/CD45/CD20.

For surface staining, approximately 3 x $10^5$ cells were added to the coated wells containing the appropriate amount of fluorochrome-conjugated antibody and incubated for 15 min on ice in the dark. Subsequently, 50µL of PBS were added to each well, the tray was centrifuged at 500 g for 5 minutes and the supernatant was discarded. Next the cells were washed in 100µL of PBS, centrifuged at 500 g for 5 minutes, and the supernatant discarded. This wash step was then repeated for a total of 2 wash steps. After the last centrifugation and supernatant discard, cells were transferred to microtubes in a final volume of 250µL of PBS. The microtubes were inserted into corresponding 12 x 75 mm tubes in the loader rack of a FACScalibur flow cytometer [Becton, Dickinson (BD), San Jose, CA] for data acquisition. Daily calibration of the instrument was performed using standardized CaliBRITE Beads (BD) with FACSComp Software (BD), and compensation was performed using appropriately stained normal peripheral blood samples.

*DNA Staining*

For DRAQ5 staining, 3 x $10^5$ cells in 250µL of PBS, previously stained for surface antigens were incubated with 2µL of DRAQ5 (Biostatus Ltd., Leicesterchire, UK) for 5 minutes at room temperature protected from bright light as previously described [5].

*Data Acquisition*

Cells were acquired on a FACSCalibur 4-color flow cytometer (BD) equipped with both a 488-nm argon laser and a 635-nm diode laser. The data were acquired using CellQuest software (BD). Acquisition of the whole sample was performed without gating to exclude cell doublets and debris. The total number of cells collected was 3 x $10^4$ per tube.

*Data Analysis*

For DRAQ5 DNA cell cycle assessment,

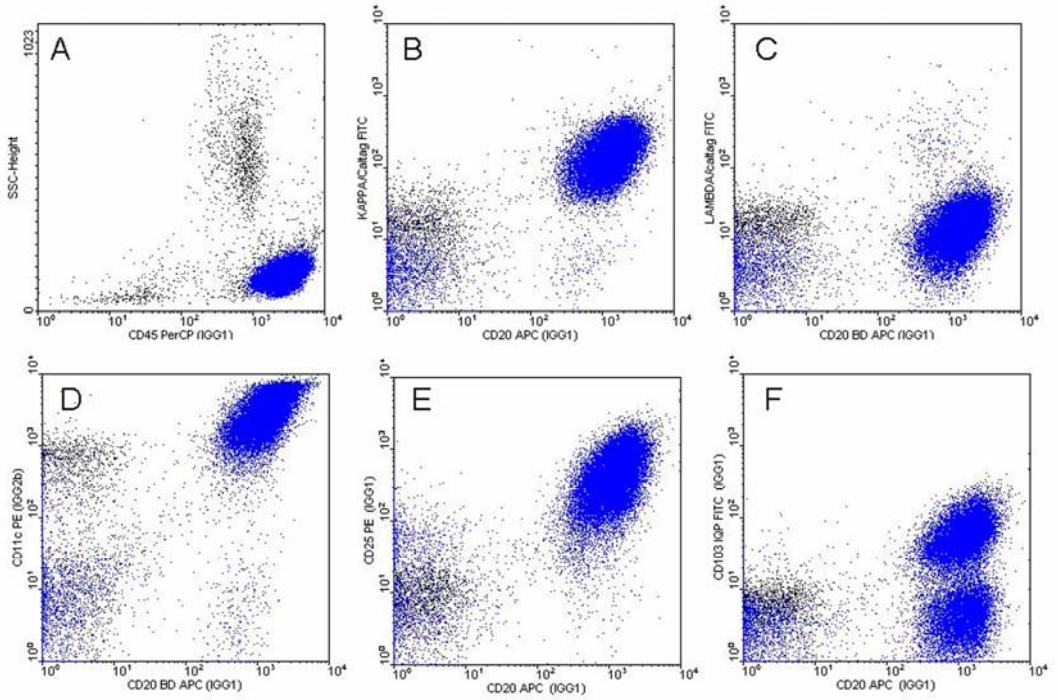


**Figure 2** Flow cytometric immunophenotyping of the bone marrow aspirate shows a predominant population of abnormal lymphocytes (shown as blue events) with high density CD11c, CD20 and CD25 expression and kappa light chain restriction (**A**-**E**). These cells display an unusual pattern of CD103 expression with approximately half of the population being negative for CD103 (**F**).

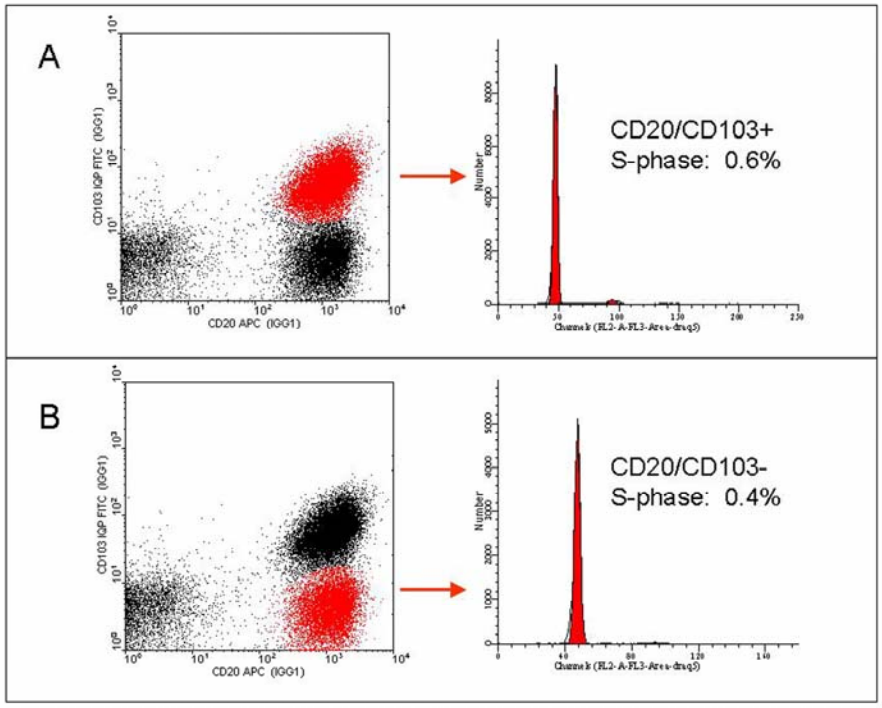


**Figure 3** DNA cell cycle analysis. In order to determine if differences in tumor proliferation were present between the CD103+ and CD103- tumor cells, simultaneous single tube DNA analysis was performed using CD20/CD103/DRAQ5 DNA dye. Based on the CD20/CD103 expression, DRAQ5-derived DNA analysis was performed by gating on the CD20+/CD103+ (**A**) and CD20+/CD103- (**B**) hairy cell populations. In panel **A**, the DNA analysis represents the gated CD20+/CD103+ population (in red). Likewise, the DNA analysis in panel **B** represents the gated CD20+/CD103- population (in red). DNA analysis demonstrated a low S-phase fraction (less than 0.7%) in both populations, consistent with a low proliferative rate typical for hairy cell leukemia.

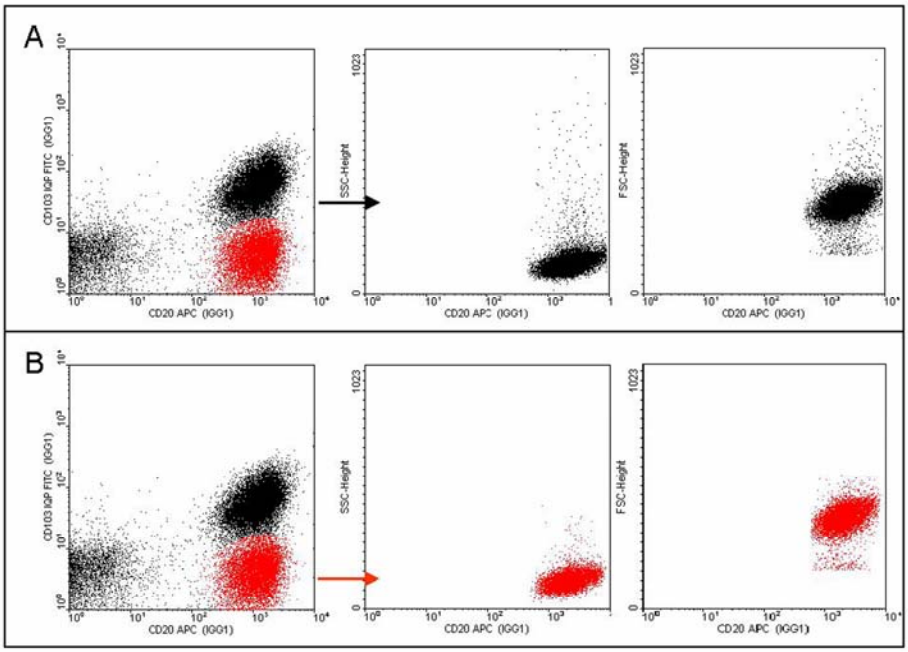


**Figure 4** Light scatter characteristics and CD20 expression. Intensity of CD20 expression, forward scatter (FSC) and side scatter (SSC) were analyzed for both the gated CD20+/CD103+ population (panel **A**, in black) and the gated CD20+/CD103- population (panel **B**, in red). Uniform intensity of CD20 expression and identical forward and side scatter characteristics were observed for both populations, consistent with a single neoplastic process.

analysis was performed on appropriate subpopulations as delineated by immunophenotype. Subpopulations were gated and analyzed for cell cycle phases using Modfit LT 3.1 software (Verity Software House, Topsham, MA). For the purposes of data display, dot plots were generated using WinMDI 2.8 (Joe Trotter, Scripps Institute).

## Results and Discussion

The immunophenotype exhibited in our case was partially characteristic of HCL. The malignant cells showed CD45 expression and side scatter properties consistent with lymphoid cells (**Figure 2A**) and exhibited a mature B-cell phenotype with restricted/clonal surface kappa immunoglobulin light chain expression. Coexpression of high density CD11c, CD25 and CD103 (**Figures 2B-F**) was consistent with HCL. In this particular case, however, a single unusual immunophenotypic characteristic was observed: CD103 expression was not present in a 50% subset of the malignant B-cells (**Figure 2F**). Variant and atypical cases of HCL have been described with varying intensity of CD11c, loss of CD25, and less commonly, lack of CD103 expression. However, the lack of CD103 in only a subset of the malignant cells in our case is an aberrant immunophenotypic finding that, to our knowledge, has not been previously reported.

It was unclear whether the cell subset lacking CD103 represented HCL, or a different B-cell neoplasm altogether. DNA analysis by flow cytometry was performed using DRAQ5 DNA binding dye to evaluate the cell cycle phases of the two separate populations. The malignant clonal B-cells of both the CD103-positive and CD103-negative cell subsets (**Figure 3**) showed a low S-phase fraction (0.6% and 0.4%, respectively) with no significant difference between the two subpopulations. The low proliferative rate was consistent with HCL [6]. Furthermore, both the CD103-positive and CD103-negative cell subsets exhibited homogeneous expression of CD20 and uniform light scatter characteristics, appearing as a single discrete cluster (**Figure 4**). In light of the kinetic, light scatter and immunophenotypic uniformity, with the lack of CD103 in a cell subset as the sole exception, the presence of a second neoplasm is highly unlikely.

CD103, a member of the integrin family of leukocyte adhesion molecules, is most commonly expressed on various subsets of human intra-epithelial lymphocytes and lamina propria T-cells [7]. CD103 is directed to the epithelial specific ligand E-cadherin and may promote homing, migration, and expansion of CD8+ T-cells in epithelial compartments in different inflammatory and autoimmune processes [8, 9], as well as in allograft rejection [10]. Although CD103 is a well-known surface marker for HCL, its biologic significance in this particular neoplasm is unknown.

Studies on HCL address the relevance of immunophenotype, or variations thereof, to clinical outcomes and response to therapy. Lack of CD25 expression in variant HCL (with variant HCL phenotypically defined as CD11c variably positive, CD25 negative, CD103 positive) is associated with poorer response to treatment with alpha interferon, splenectomy/splenic irradiation and alkylating agents [3, 4]. Additionally, treatment with nucleoside analogs such as 2-CDA and deoxycoformycin (pentostatin) may produce some response, but less effectively as compared to treatment of classic HCL [11].

Little is known about the clinical significance and response to therapy in cases of HCL exhibiting unusual expression patterns confined solely to CD103. In our case, the patient responded well to 2 cycles of 2-CDA, despite the lack of CD103 in a subset of malignant cells. The CD103 subset may not necessarily represent a separate “atypical” HCL population, but a “classic” one with transiently down-regulated CD103. A single recent report of a relapsed atypical HCL (with CD11c, CD25, and unusual CD10 expression) that was also CD103 negative achieved long term remission when treated with the nucleoside analog deoxycoformycin [12]. In contrast to the suboptimal clinical response in variant HCL lacking CD25 expression, treatment with nucleoside analogs may be fully effective in cases of HCL where the immunophenotypic variations are confined solely to CD103. The variation in CD103 expression, as opposed to defining an atypical HCL, may simply reflect a biologic spectrum of expression inherent to classic HCL (with the cause of the CD103 down-regulation currently

unknown). Moreover, changes in CD103 expression may not necessarily denote an "atypical" HCL in terms of treatment response.

In summary, we describe an unusual case of HCL expressing CD25 and CD11c, with loss of CD103 in a subset of the neoplastic cells. We demonstrate similarly low S-phase in both neoplastic subpopulations and report that clinical remission was achieved by treatment with 2-CDA.

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