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Intraclonal Heterogeneity in Concomitant Monoclonal Lymphocyte and Plasma Cell Populations: Combining Flow Cytometric Cell Sorting With Molecular Monoclonality Profiling

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Abstract

Flow cytometric cell sorting combined with molecular gene rearrangement analysis can assist in further characterizing simultaneously occurring, phenotypically distinct, monoclonal B-lymphoid and monoclonal plasma cell populations that express immunoglobulin of the same light chain. We previously established monoclonality profiles for lymphoid and plasma cell populations of lymphoplasmacytic lymphoma (LPL) bone marrow aspirates by using flow cytometric cell sorting and subsequent monoclonal gene rearrangement analysis. Our findings demonstrated that related genetic processes are less likely than unrelated genetic processes. Here, we demonstrated the utility of cell sorting combined with gene rearrangement (both immunoglobulin IgH and IgK) and IgVH sequence analysis as well as plasma cell targeted fluorescence in situ hybridization analysis in clinical cases of presumed Waldenström macroglobulinemia/LPL in which multiple distinct B-cell and plasma cell populations were identified. Combining cell sorting with subsequent molecular analysis can provide proof of identical monoclonal genotype for Waldenström macroglobulinemia/LPL and nonidentical distinct lymphoid and plasma cell populations in the clinical setting. Understanding how many clonal processes (molecular profiles) are present can help guide patient monitoring throughout treatment and potentially identify patients with worse outcomes.

Introduction

Waldenström macroglobulinemia (WM) is a chronic B-lymphoproliferative disorder associated with immunoglobulin (IgM) monoclonal gammopathy and bone marrow infiltration by lymphocytes, lymphoplasmacytic cells, and plasma cells.[1] In a previously published study, 13 (34%) of 38 specimens submitted to our laboratory for suspected monoclonal gammopathy, myeloma, or lymphoma showed identical genotypic profiles for both the B-lymphoid and the plasma cell population, whereas 25 (66%) profiles were nonidentical.[2,3] In the majority of the genotypically identical group (12 [92%] of 13), the immunophenotype was consistent with WM/lymphoplasmacytic Lymphoma (LPL)[4-6]; the WM/LPL immunophenotype was present in 16 (64%) of 25 nonidentical cases.

This article outlines the approach of sorting monoclonal plasma cells and monoclonal lymphoid cells to demonstrate the genotypic relationship between the populations in the presence of multiclonal phenotypes. The presence of multiple concomitant clonal B-cell and plasma cell populations within a specimen can blur the classification criteria of LPL and hence present a difficult diagnostic challenge. To establish the genetic relationship of concurrent clonal populations, a more detailed analysis is required than simply surface immunoglobulin light chain status alone. The combination of cell sorting and gene rearrangement analyses can distinguish between related clonal processes and 2 separate concomitant processes. This distinction can have additional findings of prognostic and/or diagnostic significance. Two case studies follow, in which we demonstrate the stepwise analysis of 3 or 4 distinct cell populations: identification by flow cytometric analysis, separation by cell sorting, and investigation with subsequent molecular and fluorescence in situ hybridization (FISH) analyses.

Results

Case 1

Three separate clonal populations were identified in a bone marrow aspirate specimen by flow cytometry in a patient with pancytopenia, monoclonal gammopathy, and lymphocytosis[7,8]; the immunophenotypes were consistent with hairy cell leukemia (HCL), chronic lymphocytic leukemia (CLL), and monoclonal plasma cells. Flow cytometric cell sorting and B-cell gene rearrangement studies along with FISH post-CD138$^+$ plasma cell selection and IgVH mutational assay studies were used to characterize each population separately (Figure 1). Both B-cell populations expressed surface lambda light chain exclusively; the monoclonal plasma cell population dem-

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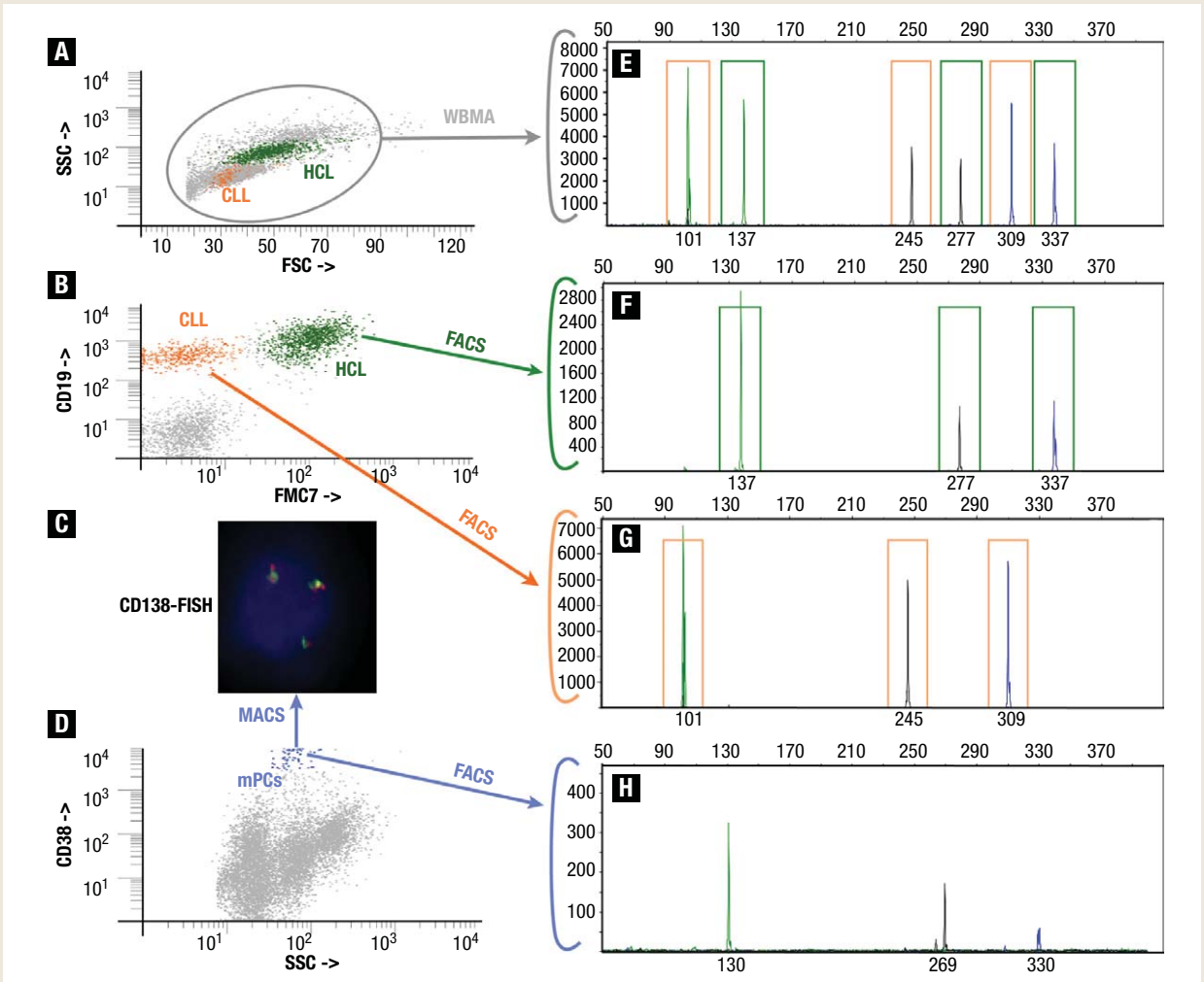
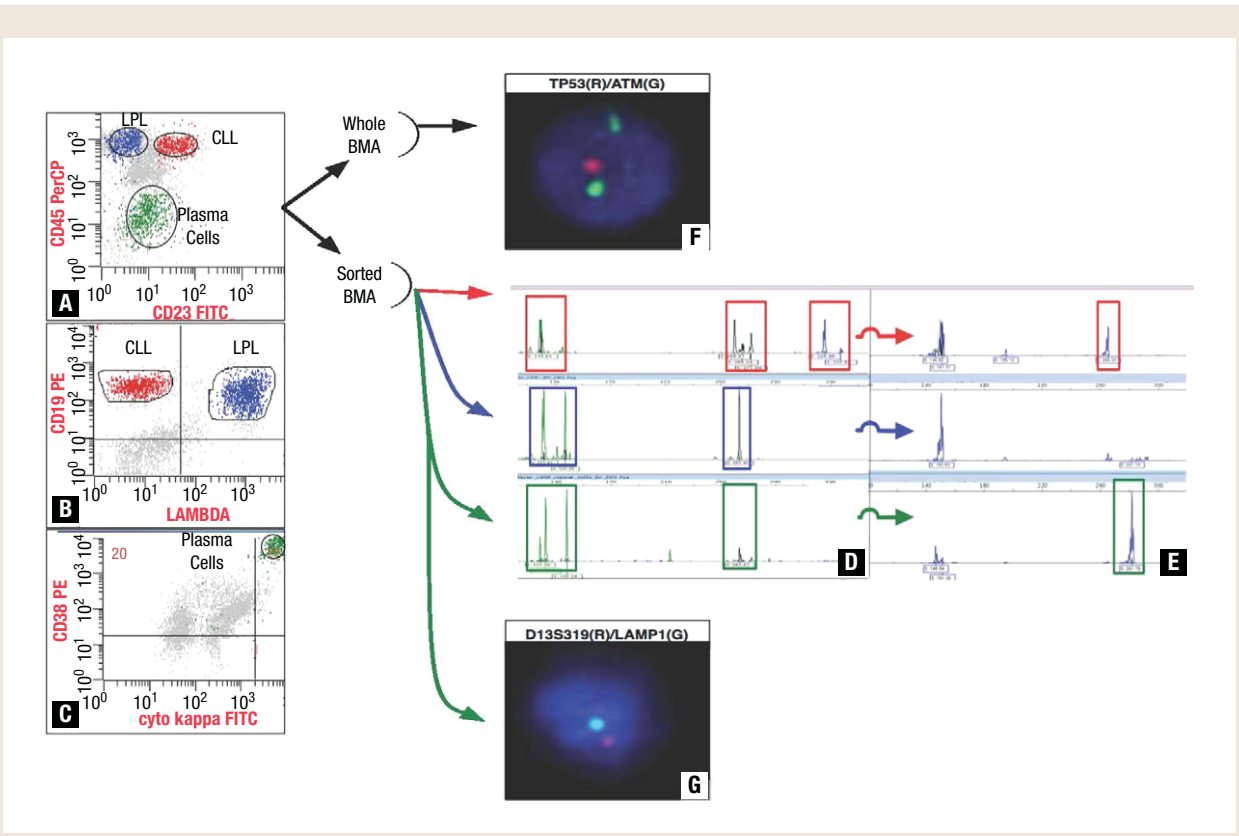
Figure 1 (A) Flow Cytometry: Histogram, Demonstrating 2 Distinct Lymphoid Populations: Complete Analysis (not illustrated) Demonstrated Immunophenotypes Compatible With Chronic Lymphocytic Leukemia (CLL) (orange) and Hairy Cell Leukemia (HCL) (green). (E) The Whole Bone Marrow Sample Was Submitted for Molecular Testing. (B) Flow Cytometry: CD19 vs. FMC7 Highlights the Distinct Population's Immunophenotypically Compatible With CLL (orange) and HCL (green). (F and G) The Flow Cytometric Sorted Cell Fractions Were Submitted for Molecular testing. (C) Fluorescence In Situ Hybridization (FISH) ($CD138^+$ cell population): MLL (11q23) Probe, Demonstrating Gain of 11q or Trisomy 11 in Plasma Cell Fraction. (D) Flow Cytometry: Histogram, Demonstrating Bright CD38 Expression in the Plasma Cell Population (blue). The Flow Cytometric Sorted Cell Fraction Was Submitted for Molecular Testing (H). (E) B-cell (immunoglobulin [Ig] H) Gene Rearrangement Studies of the Unseparate Bone Marrow Aspirate Specimen, Revealing Distinct Monoclonal Amplicons for the 3 Immunoglobulin Heavy Chain Framework Regions (IgH FR1 [blue], FR2 [black], FR3 [green]). The Biclonal Profile Detected Is Composed of the HCL (F) and the CLL (G) Fingerprint, Detected After Flow Cytometric Cell Separation. (F–H) Distinct Monoclonality Profiles With Different Amplicon Sizes for All 3 Immunoglobulin Heavy Chain Framework Regions Were Detected in the Flow Cytometric Sorted Cell Fractions. (F) HCL Population: IgH FR3 (137 bp), FR2 (277 bp), FR1 (337 bp); (G) CLL Population: IgH FR3 (101 bp), FR2 (245 bp), FR1 (310 bp); (H) Plasma Cell Population: IgH FR3 (131 bp), FR2 (269 bp), FR1 (330 bp)



onstrated kappa light chain restriction. The 3 immunophenotypically distinct populations were detected and isolated by flow cytometric cell sorting: HCL ($CD25^+$, $CD103^+$, $CD11c^+$, $FMC7^+$, increased side scatter (SSC), lambda) at 27%, CLL ($CD19/CD5^+$, low SSC, $FMC7^-$, lambda) at 11%, and monoclonal plasma cells ($CD56^-$, $CD138^+$, $CD45^{-/+}$, kappa) at 1.1%. Conventional cytogenetic analysis was essentially normal; one metaphase was detected with trisomy 8 of uncertain significance. Subsequent FISH analysis did not reveal a clonal cell population characterized by trisomy 8; however, FISH, when analyzing the $CD138^+$ enriched plasma cell fraction, revealed trisomy 11q, consistent with a plasma cell neoplasm (Figure 1C).

The 3 cell-sorted clonal cell populations were then subjected to B-cell (immunoglobulin heavy chain [IgH]) gene rearrangement studies.[9] Three genotypically unrelated monoclonality profiles were detected for the HCL, CLL, and plasma cell immunophe-

Figure 2 (A) Flow Cytometry: Histogram, Demonstrating 3 Distinct Populations: Complete Analysis (not illustrated), Demonstrating Immunophenotypes Compatible With Chronic Lymphocytic Leukemia (CLL) (red) and Lymphoplasmacytic Lymphoma (LPL) (blue), and Monoclonal Plasma Cells (green). (B) Flow Cytometry: Histogram, Demonstrating Distinct Light Chain Restriction for Both Lymphoid Populations. (C) Flow Cytometry: Histogram, Demonstrating Exclusive kappa Light Chain Expression on the Plasma Cell Population (green). (D) Immunoglobulin (Ig) H Gene Rearrangement: Monoclonal Amplicons Were Detected for the Immunoglobulin Heavy Chain Framework Regions in All 3 Sorted Cell Fractions. CLL Population (red): FR3 (118 bp), FR2 (260,271 bp), FR1 (325 bp); LPL population (blue): FR3 (122 and 137 bp), FR2 (263 bp); Plasma Cell Population (green): FR3 (122 and 137 bp), FR2 (263 bp). (E) IGK Gene Rearrangement: Distinct Unrelated Monoclonal Profiles Were Detected for the IgK Loci Primer Set in the $CD5^{+}/19^{+}$ Lymphoid population (151 bp/CLL [red]) and the $CD38^{+}$ Clonal Plasma Cell Population (282 bp [green]). Polyclonal Amplicon Distribution Was Detected in the $CD5^{-}/19^{+}$ Lymphoid Population (LPL [blue]). (F) Fluorescence In Situ Hybridization (FISH): TP53(R)/ATM (G) Probe (whole bone marrow specimen): Deletion of 17p (TP53) Deletion Was Detected. (G) FISH: D13S319(R)/LAMP1 (G) Probe Set ($CD138^{+}$ enriched cell fraction): Abnormal Signal Patterns Consistent With Monosomy 13 (or loss both 13q regions)



notype populations, respectively (Figure 1F-H). Sequencing analysis of the clonally expressed IgHV region of the HCL and CLL populations (differentiated by FMC7 expression) revealed the lack of IgHV hypermutations in both lymphoid cell fractions. In addition, sequencing studies provided further definitive evidence of unrelated genotypic profiles (VH1-46 usage in the HCL fraction vs. VH4-34 usage in the CLL fraction) (data not shown). The lack of IGHV hypermutations has been associated with poor prognosis in CLL, whereas the usage of IGHV4-34 (not identified in the HCL fraction of this patient) would confer a poor prognostic association in HCL.

Case 2

Three separate cell populations were identified by flow cytometry in a bone marrow aspirate specimen from a patient with a known clinical history of chronic lymphocytic lymphoma and WM, with macrocytic anemia and lymphocytosis. The immunophenotypes were consistent with LPL, CLL, and monoclonal plasma cells. Surface Ig light chain expression was identified as lambda for the LPL lymphocyte population and kappa for both the CLL population and the monoclonal plasma cell population. The 3 immunophenotypically distinct populations were detected and isolated by flow cytometric cell sorting: LPL ($CD19^{+}$, $FMC7^{+}$, $CD11b^{+}$, $CD5^{-}$, lambda), at 48%; CLL ($CD19^{+}/CD5^{+}$, low SSC, $FMC7^{-}$, lambda), at 29%; and monoclonal plasma cells ($CD56^{+}$, $CD138^{+}$, $CD45^{-/+}$, kappa), at 3.9%. Gene rearrangement studies, FISH post-$CD138^{+}$ enrichment, whole bone marrow FISH, and conventional cytogenetic analyses were used. Cytogenetic analysis of the whole bone marrow specimen demonstrated add (17) (p11.2) and trisomy 18 in 3 of 30 cells analyzed, consistent with either a B-cell lymphoproliferative

disorder (eg, CLL) or a non-Hodgkin lymphoma (eg, marginal zone lymphoma or LPL). Subsequent FISH analyses of the whole bone marrow specimen demonstrated a 17p (TP53) deletion (13%) (Figure 2F). Monosomy 13 (63%) was identified by FISH in the $CD138^+$ enriched cell fraction (Figure 2G), compatible with a plasma cell neoplasm.

The 3 distinct cell-sorted populations were then subjected to B-cell gene rearrangement studies by using primer sets specific for the IgH and the kappa light (IgK) chain loci. Genotypically unrelated monoclonality profiles were detected by IgH and IgK gene rearrangement studies for the 3 populations characterized immunophenotypically as LPL, CLL, and monoclonal plasma cells, respectively. Whereas, the IgH gene rearrangement analysis (left panel, Figure 2D) revealed an identical monoclonality profile for the LPL and the plasma cell fraction, flow cytometric analysis showed different light chain expression for these 2 cell populations. Subsequent IGK gene rearrangement analysis (right panel, Figure 2E) revealed the presence of a genotypically independent plasma cell population. The presence of 2 distinct plasma cell subpopulations was confirmed by detailed flow cytometric reanalysis with one population expressing cytoplasmic kappa immunoglobulin light chain, whereas the other expressed cytoplasmic lambda. These 2 populations could be discerned based on differences in light scatter properties (data not shown). The integrated interpretation of these findings confirmed the presence of 2 separate clonal plasma cell processes, one related to the LPL lymphoid cell population (consistent with the patient's known WM) and one related to an independent monoclonal plasma cell neoplasm.

Discussion

Establishing the genetic relationship of concurrent clonal populations requires detailed molecular analysis, even in cases in which the surface Ig light chains are dissimilar. The combination of flow cytometric cell sorting and molecular analysis can differentiate specimens that harbor a single related clonal process from those with 2 or more concomitant but unrelated entities. Establishing proof of an identical clonal genotype for the monoclonal plasma cell and monoclonal B-lymphoid populations can help definitively diagnose findings as WM/LPL in the setting of monoclonal lymphoid and plasma cells expressing the identical light chain. Conversely, the definitive confirmation of genotypically distinct populations may significantly improve diagnostic and prognostic classification as well as therapeutic regimens[10,11] in specimens with B-lymphoproliferative disorder(s) and a concurrent but unrelated plasma cell neoplasm. The presence of a genotypically unrelated plasma cell neoplasm may not only occur in conjunction with CLL or other lymphomas (as demonstrated in case 1) mimicking WM/LPL; an independent clonal plasma cell process may also be "hidden" within the plasma cell population already confirmed and characterized as WM/LPL (as shown in case 2).

Conclusion

The techniques demonstrated here add power to diagnostic acumen. The more specific diagnosis allows for greater accuracy in risk stratification,[12] prognostic designation, and design of appropriate monitoring strategies. Implementation of this method for routine clinical application in these diseases should be considered a valuable addition to current standard of care.

Disclosure

The authors have stated that they have no conflicts of interest.

References

1. Owen RG, Barrans SL, Richards SJ, et al. Waldenström macroglobulinemia. Development of diagnostic criteria and identification of prognostic factors. *Am J Clin Pathol* 2001; 116:420-8.
2. Zehentner BK, Cutler JA, Fritschle WK, et al. A minority of concurrent monoclonal lymphocytes and plasmacytic cells sharing light chains are genetically related in putative lymphoplasmacytic lymphoma. *Leuk Res* 2011; 35:1597-604.
3. Pantic M, Schroettner P, Pfeifer D, et al. Biclonal origin prevails in concomitant chronic lymphocytic leukemia and multiple myeloma. *Leukemia* 2010; 24:885-90.
4. Swerdlow SH, Campo E, Harris NL, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC; 2008.
5. Owen RG, Treon SP, Al-Katib A, et al. Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. *Semin Oncol* 2003; 30:110-5.
6. Harris NL, Jaffe ES, Diebold J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting—Airlie House, Virginia, November 1997. *J Clin Oncol* 1999; 17:3835-49.
7. Stelzer GT, Shults KE, Loken MR. CD45 gating for routine flow cytometric analysis of human bone marrow specimens. *Ann N Y Acad Sci* 1993; 677:265-80.
8. Terstappen LW, Johnsen S, Segers-Nolten IM, et al. Identification and characterization of plasma cells in normal human bone marrow by high-resolution flow cytometry. *Blood* 1990; 76:1739-47.
9. Treon SP, Hatjiharissi E, Merlini G. Waldenstrom's macroglobulinemia/lymphoplasmacytic lymphoma. *Cancer Treat Res* 2008; 142:211-42.
10. Shaheen SP, Talwalkar SS, Lin P, et al. Waldenstrom macroglobulinemia: a review of the entity and its differential diagnosis. *Adv Anat Pathol* 2012; 19:11-27.
11. Merchionne F, Procaccio P, Dammacco F. Waldenstrom's macroglobulinemia. An overview of its clinical, biochemical, immunological and therapeutic features and our series of 121 patients collected in a single center. *Crit Rev Oncol/Hematol* 2011; 80:87-99.
12. Gertz MA. Waldenstrom macroglobulinemia: 2011 update on diagnosis, risk stratification, and management. *Am J Hematol* 2011; 86:411-6.