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Original Article

Multidimensional Flow Cytometry for Detection of Rare Populations in Hematological Malignancies

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Abstract

Objective: Flow cytometry is becoming an important tool in the characterization of different hematological disorders. The aim of our study was to detect very rare populations in hematological malignancies using the comprehensive concept of immunophenotyping.

Patients and Methods: Six patients, including three with acute myeloid leukemia, one with acute lymphoblastic leukemia, one with myelodysplastic syndrome, and one with B-cell lymphoma, were enrolled in this study. Serial bone marrow aspirates were analyzed by three-color multidimensional flow cytometry.

Results: The core principles for the use of flow cytometric analysis are understanding deviations in abnormal antigen expression from normal cellular differentiating pathways and characterizing different patterns of antigenic aberrancy for each patient. Our results demonstrate that multidimensional flow cytometry can be used to: (1) monitor minimal residual disease after treatment; (2) aid in the differential diagnosis of cases, which are difficult to evaluate using conventional morphology; and (3) help in the staging of lymphoproliferative disorders.

Conclusion: After selecting appropriate combinations of monoclonal antibodies, and applying advanced knowledge in immunophenotyping, flow cytometry is very sensitive and specific for the detection of rare populations in hematological disorders. [*Tzu Chi Med J* 2009;21(1):40–51]

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1. Introduction

Analysis of human bone marrow aspirates is complicated by the presence of multiple cell lineages, each of which are at a variety of maturational stages. Traditionally, microscopic examination can be used to identify most hematological disorders. However, it is difficult to discriminate very rare populations of

abnormal cells from a heterogeneous marrow environment. For example, patients with fewer than 5% morphologically identifiable blasts in bone marrow samples are judged to be in hematological remission, although they may still harbor as many as 10^{11}–10^{12} leukemic cells. The use of polymerase chain reaction to identify fusion transcripts and clonal antigen-receptor gene rearrangements is attractive because of its high sensitivity of one target cell per 10^{3}–10^{6} cells. However, only a minority of hematological diseases have specific genetic abnormalities available for clinical uses.

Immunophenotyping has become a powerful tool for characterizing different kinds of hematological disorders including acute and chronic leukemias, lymphoproliferative disorders, and paroxysmal nocturnal hemoglobulinemia. Immunophenotyping can even aid as an adjunct in the diagnosis of myelodysplastic syndrome (MDS) and other myeloproliferative disorders. It has been shown that maturation of myeloid and lymphoid cells can be assessed by multidimensional flow cytometry [1–3]. This technique permits the identification of cellular lineage and maturational stage in heterogeneous cell populations, and allows the detection of potential deviations from normal cellular differentiating pathways [4–7].

The development of multidimensional flow cytometry has increased the sensitivity and specificity for the detection of minimal residual hematological malignancies after treatment. The concept of minimal residual disease (MRD) was introduced to more accurately estimate the true number of malignant cells and, in turn, to improve clinical management and curative rates. There is evidence that the presence of MRD significantly increases the rate of relapse and reduces overall survival for acute myeloid leukemia (AML) [8–11] and acute lymphoblastic leukemia (ALL) [12–14].

Here, we describe this comprehensive concept of multidimensional high resolution flow cytometry by the demonstration of six patients with various hematological diseases. The three goals of this study were: (1) to detect minimal residual disease after treatment; (2) to aid in the initial differential diagnosis of cases, which are difficult to evaluate using conventional morphology; and (3) to help in the staging of lymphoproliferative disorders.

2. Patients and methods

2.1. *Patients*

Six patients with various hematological disorders were enrolled in this analysis. Diagnostic bone marrow was obtained for morphological evaluation, cytogenetic study and flow cytometric analysis. Serial follow-up bone marrow aspirates for those patients who received treatment were also sent for analysis. All six patients signed informed consent to undergo bone marrow examination and flow cytometric tests.

2.2. *Cell preparation and multidimensional flow cytometric analysis*

The procedure for labeling cells has been previously described [15]. A working dilution of monoclonal antibodies titered for maximum fluorescence, CD45 peridinin-chlorophyll-a protein (CD45 PerCP), and 0.1 mL of well-mixed heparinized bone marrow were incubated for 20 minutes at room temperature in the dark. The erythrocytes were lysed by adding 3 mL NH_4Cl (0.83%, buffered with $KHCO_3$, pH 7.2) for 5 minutes at 37°C. The cells were then pelleted, washed twice in phosphate buffered saline (PBS), pelleted again and re-suspended in PBS with 1% paraformaldehyde.

Specimens were analyzed on a FACSCalibur (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) flow cytometer. The three color fluorescence used for monoclonal antibody conjugate included peridinin-chlorophyll-a protein (PerCP), phycoerythrin (PE), and fluorescein isothiocyanate (FITC). The instrument set-up was first standardized using lymphocytes as a reference, which was accomplished by gating on the unstained lymphocytes. This was followed by adjustment of the light scattering detectors to place the lymphocytes in a standard position in the correlative display of forward light scattering and orthogonal light scattering. Lymphocytes and maturing myeloid cells were then stained for further standardization using a series of fluorescence-conjugated monoclonal antibodies, which included CD45-PerCP, CD4-FITC and CD8-PE for lymphocytes and CD45-PerCP, CD11b-PE and CD16-FITC for myeloid cells.

For each combination of antibodies, a total of at least 10,000 events were recorded. List mode data were analyzed with WinList software (Verity Software, Tospsham, ME, USA) in accordance with previously published techniques [15–17]. For each sample, a computerized region was created to limit the analysis to viable cells with a forward and right-angle light scatter pattern characteristic of lymphocytes, monocytes, maturing myeloid elements, and blast-sized cells. Secondary gating was performed to limit further analysis to different groups of cells based on characteristic CD45 versus log side scatter properties.

Monoclonal antibodies used in this study were obtained from the indicated sources: fluorescein isothiocyanate (FITC-conjugated): CD16 from Pharmingen International (San Jose, CA, USA); CD14, CD15, CD33, CD34, CD7, CD5, CD38, HLA-DR, CD3, CD5, CD56, CD20, CD4, CD22, FMC-7, kappa, lambda, CD2 from Becton Dickinson Immunocytometry Systems