Flow Cytometric Analysis of Monocytes as a Tool for Distinguishing Chronic Myelomonocytic Leukemia From Reactive Monocytosis

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Abstract

To determine whether immunophenotypic features of monocytes are useful in differentiating chronic myelomonocytic leukemia (CMML) from reactive monocytosis, multiparameter flow cytometry was used to immunophenotype 20 bone marrow samples from patients with CMML, 10 normal marrow samples, and 20 marrow samples with reactive monocytosis. Monocytes in CMML exhibited aberrant antigen expression in all 20 cases. Abnormal antigen expression also was observed in monocytes in 11 of 20 reactive marrow samples. However, aberrant expression of 2 or more antigens was significantly less frequent in reactive monocytosis than in CMML ($P = .002$). CD56 expression with underexpression of a myeloid marker was unique to CMML monocytes. Subpopulations of monocytes with moderate levels of CD14 were present in all 3 groups. The proportion of CD14(moderate) monocytes was highest in CMML and was 20% or more in 13 of 20 CMML cases vs 3 of 20 reactive marrow samples ($P = .003$) and 2 of 10 normal marrow samples ($P = .007$). A combination of monocytosis with 2 or more immunophenotypic aberrancies with 20% or more of marrow monocytes showing moderate CD14 expression was 67% sensitive and 100% specific for CMML.

Chronic myelomonocytic leukemia (CMML) is a clonal stem cell disorder defined as a myeloproliferative/myelodysplastic disease by the World Health Organization based on the variability of the presenting WBC count, marrow cellularity, degree of dysplasia, and splenomegaly. A diagnosis of CMML requires persistent peripheral monocytosis (monocytes, $>1,000/\mu$L $[1.0 \times 10^9/L]$) and myelodysplasia or, if dysplasia is not evident, cytogenetic abnormalities or exclusion of other causes of persistent monocytosis ($\geq 3$ months).1 Although cytogenetic evidence of clonality is found in only 20% to 40% of CMML cases, dysgranulopoiesis is observed frequently.2,3 In cases lacking cytogenetic abnormalities in which morphologic dysplasia is subtle, a distinction between CMML and reactive monocytosis is diagnostically challenging.

Flow cytometric immunophenotyping has been applied widely to the diagnosis of acute leukemia and lymphoid neoplasms,4-7 but less is known of its role in diagnosis of chronic myeloproliferative disorders (MPDs) and myelodysplastic syndromes (MDSs). Clonal myeloid cells in these disorders display aberrant antigen expression.8,9 It is important to note, however, nonneoplastic granulocytes and monocytes in reactive states also might exhibit antigenic abnormalities. Previous studies of MDS and MPD primarily have used normal marrow samples for control samples (rather than pathologically altered but nonneoplastic marrow samples), and, thus, the specificity of the antigenic abnormalities is largely undocumented. In the present flow cytometric study, we compared the immunophenotypic features of monocytes in marrow samples from patients with CMML with marrow samples in reactive monocytosis and from healthy people.
Materials and Methods

We identified 20 cases of CMML from the hematopathology database at the University of Texas Southwestern Medical Center, Dallas, between October 1995 and May 2004. All cases were immunophenotyped using 3-color FACScan or 4-color FACSCalibur flow cytometry instruments with CELLQuest software (Becton Dickinson, San Jose, CA) and analyzed with Paint-a-Gate Software (Becton Dickinson). Bone marrow processing and antibody staining were performed as previously described.10 The following antibodies were used to analyze monocytes (all obtained from Becton Dickinson/Pharmingen, San Diego, CA, unless otherwise specified): anti-CD2 (55.2), CD4 (SK3), CD5 (L17F12), CD7 (4H9), CD11b (D12), CD13 (L138), CD14 (MP9), CD15 (MNA), CD16 (NK15), CD33 (P67.6), CD36 (FA6.152, Beckman Coulter, Miami, FL), CD38 (HB7), CD45 (2D1), CD56 (MY31), CD64 (10.1, Caltag, Burlingame, CA), and HLA-DR (L243). Monocytes were identified using CD45/forward and side scatter characteristics in combination with various cell surface antigens.

As control samples, 10 normal marrow samples and 20 marrow samples with 5% or more monocytes also were immunophenotyped using 4-color FACSCalibur flow cytometry. Of the 20 patients with reactive marrow monocytosis, 10 previously were diagnosed with acute lymphoblastic leukemia and 10 had a history of nonmonocytic acute myeloid leukemia; all 20 patients had been treated and had achieved complete morphologic and cytogenetic remission at the time of the study. Complete panels were performed on 15 of 20 marrow samples containing CMML and on all normal and reactive marrow samples.

Positive antigen expression was defined as at least 20% of the monocytic population showing fluorescence above the background isotypic control staining for the same cell population. Aberrant monocyte immunophenotypes of CMML or reactive marrow samples were defined as at least a half-log shift of the monocyte population (decreased or increased antigen expression) compared with monocytes in normal marrow samples. Levels of CD14 on marrow monocytes were defined as strong positive or moderate positive if beyond or below the upper level of CD14 on granulocytes, as determined in a CD14/CD38 dot plot in Image 2I.

Image 1I Cluster analysis of monocytes using CD45/side scatter and forward/side scatter characteristics. A, A representative normal bone marrow sample. B, A representative marrow sample from a patient with chronic myelomonocytic leukemia. Green, granulocytes; blue, monocytes; light blue, small lymphocytes.
Statistical analysis was performed using SPSS statistical software (SPSS, Chicago, IL). Categorical variables were analyzed with a 2-tailed $\chi^2$ test. Continuous variables were analyzed with independent-sample $t$ tests.

**Results**

**Normal Bone Marrow**

Immunophenotypic analysis of monocytes in the 10 normal marrow samples demonstrated the following immunophenotype: positive for CD4, CD13, CD15, CD33, CD36, CD38, CD45, CD64, and HLA-DR; variably but predominantly bright positive for CD11b and CD14; predominantly negative for CD16; and negative for CD2, CD5, CD7, and CD56. Characteristic patterns of antigen expression in normal monocytes are shown in [Image 3]. Because expression levels of CD11b, CD14, and CD16 varied in the monocytes and could increase or decrease during monocytic maturation and activation, they were not included for the assessment of immunophenotypic aberrancy. The proportion of monocytes that were CD14(moderate) ranged from 4.2% to 29% (mean ± SD, 11% ± 7%) and was 20% or more in 2 of 20 cases. Morphologic examination revealed that the monocytes were predominantly mature. A minority population of less mature monocytes was present in some cases, characterized by a more compact nuclear shape, slightly more dispersed chromatin, occasional small nucleoli, and less abundant cytoplasm. Cells with the cytologic features of neoplastic promonocytes were not seen.

**Reactive Monocytosis**

Monocytes in reactive marrow samples ranged from 5% to 14% (mean, 7.5%) of events. In CD45 and scatter plots,
they showed a distribution similar to that of normal marrow monocytes (Image 1). An aberrant immunophenotype in monocytes was found in 11 cases, including expression of CD56 (6), and underexpression of HLA-DR (7) and CD13 (1) (Table 1). Of 20 cases, 3 (15%) exhibited 2 or more aberrancies; the combinations of the aberrancies were CD56+/HLA-DR(partial +) (2 cases) and CD2+/HLA-DR(partial +) (1 case). The proportion of monocytes that were CD14(moderate) varied from 1% to 28% (mean ± SD, 12% ± 7%) and was not significantly different from that seen in normal marrow samples (P = .65). Of 20 cases, 3 (15%) showed 20% or more CD14(moderate) monocytes. Morphologically, the monocytes showed a spectrum of maturation with a predominance of more mature forms; the number of morphologically immature monocytes varied. Cells with the cytologic features of neoplastic promonocytes again were not seen.

Chronic Myelomonocytic Leukemia

Monocytes in the marrow samples containing CMML ranged from 5% to 42% (mean, 18%) of events. They frequently
showed a partial overlap with granulocytes in a forward and side scatter plot owing to downward shift of hypogranular granulocytes but were well separated from granulocytes in a CD45/side scatter plot (Image 1). At least 1 aberrancy of monocytes was observed in all 20 cases (Table 1); the aberrancies included underexpression of HLA-DR (10 cases), CD13 (3 cases), CD15 (1 case), or CD36 (1 case), and expression of CD56 (16 cases) or CD2 (2 cases) [Image 4]. Of 15 cases of CMML analyzed with complete panels, 10 (67%) exhibited 2 or more aberrancies in the monocytes compared with 3 of 20 marrow samples with reactive monocytosis (P = .002) [Table 2]. Compared with the monocytes in reactive marrow samples, the combination of aberrant expression of CD56 and underexpression of a myeloid antigen was unique to CMML, although it was seen in only 8 (40%) of 20 cases (Table 1).

The fraction of CD14(moderate) monocytes in CMML ranged from 2.3% to 42% (mean ± SD, 22% ± 10%) and was significantly higher than in reactive (P = .003) and normal (P = .007) marrow samples [Figure 1]. At least 20% of monocytes expressed moderate levels of CD14 in 13 (65%) of 20 CMML marrow samples. The combination of 2 or more immunophenotypic aberrancies on monocytes and 20% or more of marrow monocytes expressing moderate CD14 was found in 67% of CMML cases but in no normal or reactive marrow samples (each P < .001).

Myeloid dysplasia was identified in 17 of 20 cases of CMML by morphologic examination. In the remaining 3 cases without overt dyspoiesis, 1 showed 2 aberrancies in monocytes with 34% CD14(moderate) monocytes; 1 showed 1 aberrancy in monocytes with 42% CD14(moderate) monocytes; and 1 case was analyzed with a limited panel, precluding a complete assessment of monocyte immunophenotype. Dysplastic changes in monocytes were subtle and identified only in occasional cases of CMML [Image 5], whereas a shift of monocytic maturation toward immaturity was observed in the majority of cases.

**Discussion**

Flow cytometry has emerged as a vital tool in the diagnosis of hematolymphoid tumors because of its rapid turnaround time and its ability to simultaneously analyze multiple antigens on individual cells, distinguish and quantify many distinct populations and subpopulations, and provide reproducible assessments of relative levels of antigen expression. The role of flow cytometry in the diagnosis and classification of acute leukemia and lymphoid neoplasms is well established, but its usefulness in the diagnosis of MDSs and MPDs is less well defined. In part, this is due to the complex patterns of antigen expression observed in normal maturing and reactive myeloid populations.8,11

The diagnostic hallmark of CMML is blood monocytosis, which also can occur in a wide variety of nonneoplastic conditions. In scenarios in which cytogenetic abnormalities are absent and dysplasia is not prominent, the reliable distinction of CMML from reactive monocytosis can be challenging. Additional diagnostic tools to aid in this differential diagnosis would be highly desirable. To this end, we studied the application of multiparameter flow cytometry in the diagnosis of CMML.

Previous flow cytometric studies of chronic MPDs and MDSs primarily have used normal marrow samples as control groups. However, because a variety of antigenic alterations may be observed in reactive granulocyte and monocyte populations, it is critical to include examples of reactive states as additional control groups to ensure the specificity of aberrations observed in neoplastic myeloid disorders. We found that monocytes in marrow samples from patients with reactive monocytosis frequently exhibited abnormal immunophenotypes compared with normal marrow monocytes. Most common were decreased HLA-DR and expression of CD56.

**Table 1**

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* Disease-free patients who were treated for acute lymphoblastic leukemia.
† Disease-free patients who were treated for acute myeloid leukemia.
‡ Cases immunophenotyped with limited panels only.
Xu et al / IMMUNOPHENOTYPING OF CMML

Altered immunophenotypes in nonneoplastic monocytes are not well documented, but decreased HLA-DR on circulating monocytes has been reported in posttrauma patients.12-14 Reduction in HLA-DR expression may be regulated by cytokines and may be partly responsible for impaired immune responsiveness to infection in these patients.15,16 Aberrant expression of CD56 on monocytes and granulocytes has been documented in MDS and monocytic leukemia.17,18

A recent study demonstrated a minor CD56(low) monocyte population (0.16%-3.5%; mean ± SD, 1.3% ± 1%) in the peripheral blood of healthy individuals.19 Karandikar et al10 observed CD56 expression on monocytes in patients with Down syndrome. In the present study, CD56 was expressed by reactive marrow monocytes in 7 of 20 disease-free patients who had received previous chemotherapy for leukemia. Thus, CD56 expression on monocytes is not specific for neoplasia and must be interpreted cautiously and in the context of other clinicopathologic findings.

Although alteration of 1 antigen on monocytes was observed in 55% of the reactive monocytoses, abnormal expression of 2 or more antigens on the monocytoses was seen in only 15% of the cases. In contrast, monocytes in CMML exhibited 2 or more aberrancies in two thirds of cases. These aberrancies included decreased expression of monocytic associated antigens CD13, CD15, CD36, and HLA-DR and aberrant expression of nonmyelomonocytic antigens CD2 and CD56. Furthermore, the combination of CD56 expression and underexpression of a myeloid antigen was specific for CMML but only 40% sensitive. Partial loss of CD13,
CD14, and CD15 and expression of CD56 on monocytes have been reported in CMML.18

Bright expression of CD14 is highly specific for the committed monocyte/macrophage lineage.20 However, subpopulations of presumably immature monocytes are seen in bone marrow with moderate CD14 expression, similar to the levels seen in normal granulocytes. Because of this, diminished CD14 expression per se was not defined as an immunophenotypic aberration in our study. However, the proportion of CD14(moderate) monocytes was analyzed in each case to provide a quantitative comparison of this population in the 3 groups. A significantly higher proportion of CD14(moderate) marrow monocytes was found in CMML than in reactive monocytosis and normal marrow samples. The CD14(moderate) monocytes expressed dimmer CD45 than CD14(strong) monocytes, supporting the interpretation that they represent relatively immature cells. Furthermore, the combination of monocytosis with 2 or more immunophenotypic aberrancies and 20% or more CD14(moderate) marrow monocytes was 67% sensitive and 100% specific for CMML.

In this study, we focused exclusively on the immunophenotypic features of monocytes. Immunophenotypic aberrations of myeloblasts and maturing granulocytes also have been documented in MDS and chronic MPD.8,9 For example, blasts may express nonlineage antigens such as CD56 and CD7, show asynchronous expression of CD34 and maturation antigens such as CD11b and CD15, or underexpress or overexpress normally expressed antigens such as CD34, CD117, and HLA-DR. Similarly, granulocytes may show disturbances in the normal qualitative pattern of maturation, show decreased orthogonal light scatter, or aberrantly express antigens such as CD56. The granulocytes in some cases in the present series manifested decreased orthogonal light scatter, suggesting hypogranularity. Thus, it is possible that a broader assessment of immunophenotypic aberrations in other myeloid populations might further enhance the distinction of neoplastic and reactive monocytoses. However, it is imperative that the specificity of these various immunophenotypic aberrancies be tested against appropriate control groups (eg, nonneoplastic cytopenias, regenerating marrow samples).
Our data indicate that assessment of the immunophenotypic features of monocytes can aid in the distinction of CMML from reactive monocytosis. Although individual abnormalities are nonspecific, the combination of 2 or more aberrancies and 20% or more CD14(moderate) monocytes is 67% sensitive and completely specific for CMML.

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References


