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Letter to the Editor

Two Granulocytic Regions in Bone Marrow with Eosinophilia Evaluated by Flow Cytometry

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TO THE EDITOR

Flow cytometry (FCM) with a CD45 gate is widely used for analyzing phenotypes of bone marrow cells in hematological disorders. This method clearly separates bone marrow cells into erythroblasts, lymphocytes, monocytes, myeloblasts, and granulocytes.[1] Each cell group shows a distinct distribution characterized by the CD45 intensity and side scatter properties. Usually, granulocytes show a single population of cells with bright CD45 and high side scatter properties. In some situations, the granulocytic region in the bone marrow is divided into two adjacent regions, although causal events are not known.[2] We report that bone marrow granulocytic regions in eosinophilia are separated into two regions by FCM.

From March 2008 to October 2013, bone marrow aspiration was performed in 12 patients with eosinophilia. The obtained bone marrow cells underwent smear examination, chromosomal analysis including fluorescence *in situ* hybridization analysis, and FCM as routine practice. In the bone marrow smear examinations, eosinophils were distinguished between mature and immature forms. Mononuclear cells were separated from an aliquot of the bone marrow samples and used for FCM with a CD45 gate, as previously reported.[3] Initially, FCM was performed to analyze phenotypes of blasts in the blast region. In this study, FCM data were reevaluated by setting a gate for identifying granulocytes characterized by bright CD45 expression and high side scatter properties (Fig. 1A). Phenotypes of the cells were analyzed using a flow cytometer (FACSCalibur or FACSCanto II; BD Biosciences, San Jose, CA, USA). For reanalyzing the FCM data, neither informed consent from each patient nor permission from the Institutional Review Board of our hospital was obtained.

Table 1 shows the characteristics of the patients. The median age was 56, with 9 males and 3 females. All patients showed more than 1,000/μL of eosinophils in the peripheral blood. The rates of immature and mature eosinophils in the bone marrow (mean ± SD) were 15.2 ± 9.2% and 22.6 ± 12.2%, respectively. Karyotypic abnormalities were not shown in the analyzed samples. All patients was diagnosed as having secondary eosinophilia as follows: graft-versus-host disease after hematopoietic stem cell transplantation, 3 patients; drug allergy, 2; asthma, 1; liver cirrhosis, 1; chronic renal failure, 1; myocardial infarction, 1; erythroderma, 1; cardiomyopathy, 1; and unknown etiology, 1. As shown in Fig. 1B, two distinct granulocytic regions, G1 and G2, are observed in bone marrow with eosinophilia, compared with normal bone marrow. According to the CD45 intensity and side scatter properties, the G2 region corresponds to the neutrophilic granulocyte region. The percentages of cells in the G2 region and immature myeloid cells including myeloblasts to metamyelocytes evaluated by smears were 19.3 ± 5.9% and 10.3 ± 7.5%, respectively. As shown in Fig. 2, the antigen expression profiles in the G2 region are consistent with those in neutrophilic granulocytes along with the neutrophilic differentiation process, that is, from the stage of promyelocytes, disappearance of HLA-DR expression, increasing expression of CD11b and CD15, and fluctuating expression of CD13 and CD33.[2] The G1 region shows higher CD45 intensity than the G2 region and equal side scatter properties to the G2 region (Fig. 1B). The antigen expression profiles in the G1 region were different from those in the G2 region to some extent (Fig. 2). The G1 region showed higher positivity for CD10, CD11b, CD13, CD25, CD36, and HLA-DR and lower positivity for CD33 than the G2 region. In eosinophils, CD11b, CD25, and HLA-DR expression is upregulated by stimuli.[4,5] In neutrophilic granulocyte differentiation, HLA-DR is ex-

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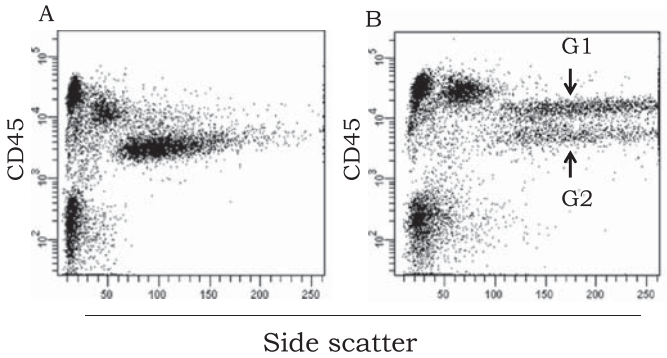

Fig. 1. Flow cytometric analysis of bone marrow mononuclear cells. (***1A***) Normal bone marrow from a bone marrow transplantation donor. (***1B***) Bone marrow with eosinophilia from a patient with graft-versus-host disease. *G1* and *G2* indicate two separated granulocytic regions.

Table 1. Patients' characteristics

Clinical data	Patients
Age (years)[#]	56 (23-81)
Sex (male/female, No.)	9/3
Disease (No.)	
Secondary eosinophilia	12
White blood cell counts (/μL)[§]	17,492 ± 10,314
Eosinophils (%)[§]	43.4 ± 20.9
Eosinophils (/μL)[§]	7,927 ± 5,891
Eosinophils > 1,000/μL (No.)	12
Eosinophils > 1,500/μL (No.)	10
Hemoglobin (g/dL)[§]	10.4 ± 2.9
Platelet count ($\times 10^4$/μL)[§]	21.4 ± 13.1
Bone marrow eosinophils	
Immature (%)[§]	15.2 ± 9.2
Mature (%)[§]	22.6 ± 12.2

[#], median; [§], mean ± SD; No., total number. Peripheral blood count was conducted on the day of bone marrow examination or another day near to it.

pressed only at the stage of myeloblasts, and CD25 is not expressed in any stage.[2] The bone marrow mononuclear cells used in this study contain immature myeloid cells. Taking these findings together, the G1 region is suggested to contain immature eosinophils. The percentages of cells in the G1 region and immature eosinophils evaluated by smears were 8.6 ± 7.2% and 9.2 ± 6.9%, respectively. The ratio of the G1 and G2 regions was 0.45, while the ratio of immature eosinophils and immature myeloid cells was 0.89. The difference of the ratios between the two measurements may be due to the cell source, namely, FCM for mononuclear cells and morphological examinations for whole cells. For FCM analysis, ammonium chloride-lysed whole bone marrow cells are frequently used instead of bone marrow mononuclear cells.[6] FCM using whole cells is superior in comparison with the results of morphological examinations. The differences of the antigen expression profiles between normal neutrophilic granulocyte differentiation and normal eosinophil differentiation have been insufficiently examined, except for CD10 and CD16.[6] The two antigens are expressed only from the stage of metamyelocyte/band cells; however, they are not expressed in mature eosinophils.[2,6] Because CD36 is not expressed in any granulocytes, including eosinophils, but is expressed in monocyte/macrophage-lineage cells and erythroblasts,[2] it is not clear why the G1 region contains CD36-positive cells. In this study, patients with hypereosinophilic syndrome were not included. It is necessary to confirm that the G1 region exists in the bone marrow in cases of this syndrome. Using multicolor FCM and a cell sorting technique for the samples of bone marrow with eosinophilia, it should become clear whether or not cells in the G1 region are immature eosinophils.

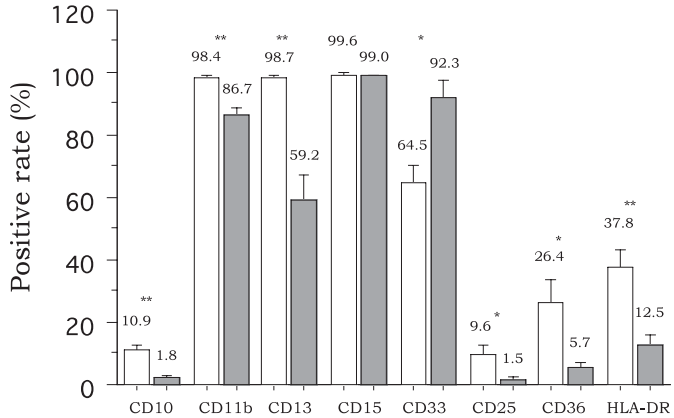

Fig. 2. Antigen expression in the G1 and G2 regions. The *white* and *gray bars* indicate the G1 and G2 regions, respectively. The data show the mean ± SD.
*, $p < 0.05$; **, $p < 0.01$

REFERENCES

1 Stelzer GT, Shults KE, Loken MR: CD45 gating for routine flow cytometric analysis of human bone marrow specimens. Ann N Y Acad Sci 677:265-280, 1993

2 Gorczyca W: Flow cytometry in neoplastic hematology (Morphologic-immunophenotypic correlation), 2nd ed, Colchester, Informa Healthcare, pp.51-57, 2010

3 Muroi K, Fujiwara S, Tatara R, Sugimoto M, Yamamoto C, *et al.*: CD56 expression in normal immature granulocytes after allogeneic hematopoietic stem cell transplantation. J Clin Exp Hematop 53:247-250, 2013.

4 Rothenberg ME, Hogan SP: The eosinophil. Annu Rev Immunol 24:147-174, 2006

5 Isogai S, Hayashi M, Yamamoto N, Morishita M, Minezawa T, *et al.*: Upregulation of CD11b on eosinophils in aspirin induced asthma. Allergol Int 62:367-373, 2013

6 van Lochem EG, van der Velden VH, Wind HK, te Marvelde JG,

Westerdaal NA, *et al*.: Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: reference patterns for age-related changes and disease-induced shifts. Cytometry B Clin Cytom 60:1-13, 2004