Phenotypic Difference of Normal Plasma Cells From Mature Myeloma Cells

By Hironori Harada, Michio M. Kawano, Naihui Huang, Yuka Harada, Koji Iwato, Osamu Tanabe, Hideo Tanaka, Akira Sakai, Hideki Asaoku, and Atsushi Kuramoto

We have recently shown that two-color analysis with fluorescein isothiocyanate (FITC)--anti-CD38 antibody could clearly distinguish myeloma cells (plasma cells) from other hematopoietic cells in the bone marrow. Myeloma cells (plasma cells) alone were located at CD38+ strongly positive (+ + +) fractions. To further distinguish normal plasma cells from mature myeloma cells phenotypically, we examined immunophenotypes of normal plasma cells and myeloma cells by two-color flow cytometry with FITC--anti-CD38 antibody and phycoerythrin staining with antibody to VLA-4, MPC-1, CD44, CD56, CD19, CD20, CD24, or CD10. Normal plasma cells were all VLA-4+ VLA-5+ MPC-1+ CD44+ CD19+ CD56− in the bone marrows from seven healthy donors, tonsils from four patients with chronic tonsillitis, a spleen from one patient with idiopathic thrombocytopenic purpura, and lymph nodes from two patients with chronic lymphadenitis, respectively. On the other hand, mature myeloma cells (12 of 20 cases), VLA-4+ VLA-5+ MPC-1+, were all CD19− and most of them CD56−, and there were no myeloma cells with the CD19+ CD56− phenotype in the 20 cases of myelomas we tested. Thus, as for the expression of CD19 and CD56, normal plasma cells from various tissues are all CD19− CD56−, whereas no myeloma cells have the CD19+ CD56− phenotype. According to this finding, we investigated the expression of CD19 and CD56 on plasma cells (CD38+ + fractions) in monoclonal gammopathy of undetermined significance (MGUS). Both CD19+ CD56− and CD19+ CD56+ plasma cells were found in all five cases of MGUS we tested, suggesting that MGUS consists of phenotypically normal plasma cells and myeloma cells. Therefore, it is reasoned that phenotypic analysis of plasma cells with anti-CD19 and anti-CD56 antibodies can distinguish normal plasma cells from malignant plasma cells (myeloma cells), and can detect malignant plasma cells even in MGUS or premylema states.

MATERIALS AND METHODS

Patients. BM aspirations were performed in 7 healthy donors, 20 myeloma patients, and 5 MGUS patients. Fifteen myeloma patients were IgG type, 3 IgA type, 1 IgD type, and 1 Bence-Jones (BJ) type; and five were in the clinical stage I, 4 in stage II and 11 in stage III. Two MGUS patients were IgG and three were IgA.

Tonsils from four patients with chronic tonsillitis, a spleen from one patient with idiopathic thrombocytopenic purpura (ITP), and lymph nodes from two patients with chronic lymphadenitis were also studied. Informed consent was obtained from all patients before these procedures.

Two-color flow cytometry. BM mononuclear cells were freshly isolated from BM aspirates by Ficoll-Hypaque density gradient centrifugation as described previously. Tonsils, spleen, or lymph nodes were finely minced, and the cells were suspended in RPMI 1640 medium (Nissui, Tokyo, Japan) supplemented with 10% fetal calf serum (FCS; M.A. Bioproducts, Walkersville, MD). The cells (5 × 10⁷ cells) were stained at 4°C for 30 minutes with anti-CD10 (CALLA), anti-CD19 (Leu-12) antibody (Becton Dickinson, San Jose, CA); anti-CD20 (B1), anti-CD56 (NK1-1) antibody (Coulter Immunology, Hialeah, FL); anti-CD24, anti-CD44, anti-VLA-4, anti-VLA-5 antibody (Immunotech S.A., Marseille, France); or MPC-1 antibody that we established and that recognized normal plasma cells and mature myeloma cells but not immature myeloma cells. Anti-CD10, anti-CD19, and anti-CD56 antibodies were also purchased from Immunotech S.A. After washing, the cells were incubated with phycoerythrin (PE)-labeled goat antimouse IgG (Immunotech S.A.) at 4°C for 30 minutes. The cells were then washed and subsequently incubated with normal mouse IgG to block nontpecific binding at 4°C for 20 minutes. Subsequently, the cells were incubated with FITC-conjugated anti-CD38 antibody (Immunotech S.A.) at 4°C for 30 minutes. Immunofluorescence of the membrane was evaluated by a
flow cytometer (Cytron; Ortho Diagnostic Systems, Raritan, NJ). Two-color flow cytograms with FITC-anti-CD38 (x-axis, log scale) and PE-staining (y-axis, log scale) were presented.

Cell sorting. The cells were stained with FITC-anti-CD38 and PE-staining as described above. Cell sorting was performed by a cell sorter (FACS-IV; Becton Dickinson). The sorted cells were cytospun and stained with Wright's solution.

RESULTS

Mononuclear cells were isolated from BM, tonsils, spleen, or lymph nodes, and stained with FITC-anti-CD38 and PE—anti-VLA-5 antibody. The CD38++ cells were sorted by a cell sorter (FACS-IV) and stained with Wright's solution. In healthy donors, normal plasma cells were located at the CD38++ fractions. The cells sorted from CD38++ fractions were all plasma cells morphologically, as shown in Fig 1A, but the cells sorted from CD38− or CD38weak positive (+) contained almost no plasma cells. In myeloma patients, the cells with CD38++VLA-5− were also sorted and their morphology was examined under microscope. Figure 1B shows that the cells sorted from CD38++VLA-5− were morphologically mature myeloma cells according to Greipp's classification.15 CD38++ fractions and CD19+B cells in the BM existed in the mononuclear cell fractions (Fig 2A), but not in the other remaining fractions (Fig 2B) of the forward and right scattering profile. The percentage of myeloma cells or plasma cells in BM mononuclear cells based on morphologic examination were almost the same as that of CD38++ fractions by flow cytometry. Therefore, it was confirmed that normal plasma cells as well as myeloma cells were located at the CD38++ fractions on two-color cytogram with FITC-anti-CD38 antibody staining.

The phenotypes of myeloma cells from 20 myeloma patients were studied (Table 1). Myeloma cells from patients no. 1 through 12 showed their phenotype to be VLA-4+VLA-5−MPC-1− and were all mature myeloma cells, whereas myeloma cells from patients no. 13 through 20 were immature myeloma cells (VLA-4−VLA-5−MPC-1−), as demonstrated recently.16

As for expression of CD56 and CD19, myeloma cells in most patients (13 of 20 cases), mature and immature myeloma cells, were CD56−CD19−, as shown in Fig 3B; myeloma cells in 5 cases (myeloma patients no. 1, 3, 6, 8, and 17 in Table 1) were CD56−CD19+, as shown in Fig 3A; and myeloma cells in 2 cases (patients no. 13 and 14 in Table 1) were CD56+CD19+. However, there were not any myeloma cells with their phenotype of CD56+CD19−.

Normal plasma cells were harvested from BM aspirates in 7 healthy donors, from tonsils of 4 patients with chronic tonsillitis, from the spleen of a patient with ITP, from the lymph nodes of two patients with chronic lymphadenitis, as described in Materials and Methods. Normal plasma cells in various tissues were all VLA-4−VLA-5−MPC-1−CD44+CD56−CD19− (Table 1). Two-color cytograms of normal plasma cells were shown in Fig 3: Fig 3D represents a cytogram of plasma cells from the BM, Fig 3E represents that of plasma cells from the tonsil, and Fig 3F represents that of plasma cells from the spleen.

Furthermore, the expression of other B-cell-lineage antigens was also examined on normal plasma cells. All of the normal plasma cells we tested showed the CD20+CD22+CD10+ phenotype (Table 1). This phenotype was almost the same as that of myeloma cells, although there were only a few myeloma cells with the CD20+ phenotype.

Our data clearly show that the CD38++CD56+CD19− phenotype is found only on normal plasma cells in various tissues such as BM, tonsils, spleen, and lymph nodes, because there were no myeloma cells with the CD38++CD56+CD19− phenotype in myeloma patients.

Additionally, we examined the expression of CD19 and CD56 on plasma cells in 5 cases of MGUS (Table 1). Plasma...
cells (CD38++ fractions) from 3 patients showed a large number of mature VLA-4+VLA-5+MPC-1+ and a few immature VLA-4+VLA-5+MPC-1-. Those from the other 2 patients consisted of mature VLA-4+VLA-5+MPC-1+ alone. As for expression of CD56 and CD19, plasma cells in 4 of 5 patients consisted of both CD56+CD19+ and CD56+CD19- cells (Fig 3C). CD19+ cells sorted by a cell sorter were all CD56+ cells, whereas sorted CD19- cells were all CD56- cells (data not shown). One patient showed that plasma cells had both CD56-CD19+ and CD56-CD19- cells. Thus, these data indicate that normal plasma cells with CD56-CD19+ and malignant plasma cells (myeloma cells) are detected in MGUS.

**DISCUSSION**

In this report, we clearly show that normal plasma cells have the phenotype of CD38++VLA-4+VLA-5+MPC-1+CD44+CD56-CD19-CD20-CD24-CD10- by two-color staining with FITC-anti-CD38 antibody. Mature myeloma cells are morphologically as mature as normal plasma cells. However, with regard to the expression of CD56 and CD19, mature myeloma cells are clearly distinguished from normal plasma cells (CD56+CD19+). Most mature myeloma cells are CD56+CD19+, although a few myeloma cells with CD56+CD19+ or CD56+CD19- are found. As for the expression of CD56 (natural killer [NK] cell-associated marker), Van Camp et al8 have already documented that myeloma cells in most of the myeloma patients (43 of 55 cases) were CD56+, but there were no plasma cells with CD56+ phenotype either from 23 MGUS patients or from normal 10 BMs, 8 lymph nodes, and 3 tonsils. Our data support their findings. However, they did not refer to the expression of CD19 on normal plasma cells or mature myeloma cells. Phenotypic analysis with both anti-CD56 and anti-CD19 antibodies can clearly distinguish normal plasma cells from mature myeloma cells.

The CD56 antigen is reported to be expressed on NK cells and a subset of T cells.9 Recent studies showed that the expression of CD56 was also found on myeloid leukemia cells, trophoblast cells, sea urchin coelomonocytes, small cell lung cancer cells, and neural tissues.10,11 Conversely, CD56 antigen is also found to be identical to the 140-Kd isoform of N-CAM20 and to be the adhesion molecule that binds each other (homophilic binding).21 The biologic role of CD56 molecule expressed on most myeloma cells, but not on normal plasma cells is still unclear. However, it is possible that CD56 molecule may be involved in the interaction between myeloma cells and BM stromal cells. BM stromal cells were recently shown to express CD56 antigen22 and CD56 is one of the adhesion molecules that induce homophilic adhesion, as described above. Also, it was observed that some myeloma cells attached to BM stromal cells or a stromal cell line (KM-102) and could survive for a long time.23 However, CD56 molecule alone is not enough to explain adhesion of myeloma cells to BM stromal cells, but several other adhesion molecules may be involved in such interaction. Our recent work shows that expression of one of the adhesion molecules (VLA-5) could define mature myeloma cells,18 and a monoclonal antibody (MPC-1) that we established could divide mature myeloma cells into subtypes. More mature myeloma cells were VLA-5+MPC-1+.14 VLA-5 molecule may be one of the molecules responsible for adhesion of myeloma cells to BM stromal cells. There is no apparent correlation between CD56 and VLA-5 or MPC-1 expression on myeloma cells, as shown in Table 1. Up to now, it remains unclear how myeloma cells attach to BM stromal cells or what molecules are involved in such an interaction.
### Table 1. Phenotypic Analysis of Myeloma Cells and Plasma Cells

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<th>Age</th>
<th>Stage</th>
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<th>VLA-4</th>
<th>VLA-5</th>
<th>MPC-1</th>
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<th>CD56</th>
<th>CD19</th>
<th>CD20</th>
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<td>A-λ</td>
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<td>A-λ</td>
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**Source**
- Bone marrow 1 (normal)
- Bone marrow 2 (normal)
- Bone marrow 3 (normal)
- Bone marrow 4 (normal)
- Bone marrow 5 (normal)
- Bone marrow 6 (normal)
- Bone marrow 7 (normal)

**Tonsil**
- Tonsil 1 (chronic tonsillitis)
- Tonsil 2 (chronic tonsillitis)
- Tonsil 3 (chronic tonsillitis)
- Tonsil 4 (chronic tonsillitis)

**Spleen**
- (ITP)

**Lymph node**
- Lymph node 1 (chronic lymphadenitis)
- Lymph node 2 (chronic lymphadenitis)

Abbreviations: ND, not done; MGUS, monoclonal gammapathy of undetermined significance; ITP, idiopathic thrombocytopenic purpura.

* A part of cells weakly expressed.
† Both positive and negative cells were found, and negative fraction was dominant.
‡ Both positive and negative cells were found, and positive fraction was dominant.

CD19 antigen has been considered to be the antigen of B-cell lineage and to be lost during terminal differentiation of B cells into plasma cells. Surprisingly, in this report, normal plasma cells from various tissues were all CD19+. Therefore, CD19 is one of the important markers that define normal plasma cells. Two-color analysis with FITC-anti-CD38 antibody made it possible to examine the phenotype of normal plasma cells exactly, even though there were not more than 1% plasma cells in the BM samples from healthy donors. The biologic role of CD19 expression on normal plasma cells not comprising mostly myeloma cells remains unclear.

Furthermore, it is of interest that, phenotypically, both normal plasma cells with CD56-CD19+ and malignant cells are found in MGUS. MGUS is clinically stable and biologically a low labeling index, but a part of MGUS develops malignant transformation after long periods; MGUS is considered to be a so-called premyeloma state. This finding that MGUS consist of normal plasma cells and malignant malignant cells (myeloma cells) could provide some clue as to the mechanism of malignant transformation into myeloma cells. We are now investigating the biologic and molecular difference between CD56+CD19+ and CD56+CD19- cells in MGUS.

In conclusion, we can clearly detect normal plasma cells by two-color analysis with FITC-anti-CD38 antibody and PE-anti-CD56 or PE-anti-CD19 antibody, even though very few plasma cells are present in the BM samples from plasma cell dyscrasia.
Fig 3. The expression of CD19, CD56, and VLA-5 on myeloma cells and normal plasma cells. BM mononuclear cells were freshly isolated from BM aspirates by Ficoll-Hypaque centrifugation, and tonsils, a spleen, and lymph nodes were finely minced as described in Materials and Methods. Two-color flow cytograms with FITC-anti-CD38 (x-axis, log scale) and PE-anti-CD19, PE-anti-CD56, or PE-anti-VLA-5 antibody (y-axis, log scale) are presented. The fractions (CD38 ++ ) of myeloma cells or normal plasma cells are indicated by the arrows. (A) Mature myeloma cells with their phenotype of CD19-CD56-VLA-5+ in myeloma patient no. 6 shown in Table 1. (B) Immature myeloma cells with their phenotype of CD19-CD56-VLA-5- in myeloma patient no. 18 (Table 1). (C) CD38 ++ cells in the BM from MGUS (MGUS patient no. 3 in Table 1); both CD56-CD19+VLA-5+ and CD56+CD19-VLA-5+ were present. (D) Normal plasma cells in the BM from a healthy donor (BM 1 in Table 1); CD19+CD56-VLA-5+ was found. (E) Normal plasma cells from the tonsil (tonsil 1 in Table 1); CD38+CD19+CD56-VLA-5+ was found. (F) Normal plasma cells from the spleen (spleen in Table 1); CD19+CD56-VLA-5+ was found.

We provide here the phenotypic evidence that normal plasma cells are all CD56-CD19+, and that this phenotype is not found on any myeloma cells. The presence of normal plasma cells and malignant plasma cells (myeloma cells) in MGUS will contribute to the further study on oncogenesis of myelomas.

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