Identification and Characterization of Plasma Cells in Normal Human Bone Marrow by High-Resolution Flow Cytometry

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The low frequency of plasma cells and the lack of specific cell surface markers has been a major obstacle for a detailed characterization of plasma cells in normal human bone marrow. Multiparameter flow cytometry enabled the identification of plasma cells in normal bone marrow aspirates. The plasma cells were located in a unique position in the correlation of forward light scattering, orthogonal light scattering, and immunofluorescent-labeled CD38. The identity of the sorted cell populations was confirmed by microscopic examination of Wright's stained slides and slides stained for cytoplasmic immunoglobulin using polyclonal antibodies reactive with light chains; ie, anti-κ fluorescein isothiocyanate and antiλ phycoerythrin (PE). The purity of the sorted plasma cells was greater than 97% (n = 4). The average frequency of plasma cells in normal bone marrow aspirates was low—0.25% of the nucleated cells (n = 7)—but surprisingly consistent between individuals (SD = .05; range 0.14% to 0.30%). A detailed analysis showed two distinct populations of plasma cells: (1) A population relatively smaller by forward light scattering expressed CD22, CD35, and sIgE and was identified as early plasma cells (ie, lymphoplasmacytoid), and (2) a population larger by forward light scattering lacked these markers and was identified as mature plasma cells. The antigenic profile of the normal plasma cells was determined in two-color immunofluorescence studies. The expression of cell surface immunoglobulin G (IgG), IgA, IgE, IgD, IgM, and the cell surface antigens CD10, CD11b, CD13, CD11c, CD14, CD15, CD16, CD19, CD22, CD20, CD33, CD35, CD45, and HLA-DR was determined on the plasma cells. A significant heterogeneity in cell surface antigen expression was observed within the plasma cell population. Unexpectedly, myeloid-specific cell surface antigens such as CD33 and CD13 and the early B-cell antigen identified by CD10 were expressed on a proportion of plasma cells. These observations imply that the association of myeloid and early B-cell markers described in multiple myeloma may not be associated with the neoplasia but is a normal phenomenon.

THE BONE MARROW is the site of early B-lymphocyte maturation in humans.[1] Immunoglobulin gene rearrangement, the presence of intracellular immunoglobulin (Ig), and the changes of the antigenic cell surface profile during early B-lymphocyte maturation have been extensively studied.[2-8] Four sequential maturational stages have been identified in human B-lymphocyte differentiation by means of flow cytometry, each characterized by the synchronous loss or acquisition of multiple-cell surface antigens.[9,10] Although early antigen-independent B-cell maturation is well defined, little is known about normal terminal B-cell differentiation in response to antigens. Most studies describing differentiation into plasma cells have been assessed by in vitro stimulation of B lymphocytes,[11,12] whereas characterization of normal plasma cells in situ are infrequent.[8,13] Most information regarding plasma cells has resulted from the extrapolation from malignant myelomas.[13-20]

The difficulties in identification of plasma cells as compared with B lymphocytes in normal marrow are a result of the low frequency of the plasma cells and the loss of the typical B-cell surface antigens on these cells. Although the CD38 antigen is one of the antigens expressed by human plasma cells,[15,21,22] this antigen is not specific for plasma cells and expressed on a large variety of other cells such as basophils,[23] monocytes,[23] early monomyeloid cells,[24] early B lymphocytes,[22,24] early T lymphocytes,[15,24,25] natural killer cells,[26] and activated T cells.[15]

In this study we demonstrate the use of high-resolution, multiparameter flow cytometry to identify plasma cells uniquely using their location in a three-dimensional space created by forward light scattering, orthogonal light scattering, and CD38 fluorescence intensity. The specific identification of normal plasma cells in human bone marrow permitted an extensive characterization of the antigens expressed on the normal plasma cells.

MATERIALS AND METHODS

Cell preparation. Bone marrow aspirates were obtained from consenting normal adult volunteers. For lyses of erythrocytes, 1 vol of blood was diluted with 14 vol of a lysing solution (10^{-4} mol/L EDTA, 10^{-2} mol/L $KHCO_3$, 0.17 mol/L NH_4CL in water [pH 7.3]) and gently mixed. Cells were lysed for 3 to 5 minutes and centrifuged at 200*g* for 5 minutes at room temperature. The pellet was resuspended in a volume of RPMI 1640 (Whittaker, Walkersville, MD) 14 times larger than the original bone marrow volume and centrifuged at 200*g* for 5 minutes. This washing step was repeated twice and the cells were finally resuspended in phosphate-buffered saline (PBS) containing 1% bovine serum albumin and 20 mmol/L HEPES (pH 7.3). The cell concentration was adjusted to 1×10^7/mL. Twenty microliters of pretitered monoclonal antibodies (MoAbs) were added to 100 μL of cell suspension. After incubating for 20 minutes on ice, the cells were washed once with 3 mL of the PBS solution at 4°C. The staining procedure was repeated in the succeeding step. The MoAbs used were CD16 (anti–Leu-11a fluorescein isothiocyanate [FITC]), CD19 (anti–Leu-12 FITC), CD22 (anti–Leu-14 FITC), CD20 (anti–Leu-16 FITC), HLA-DR FITC, CD45 (Hle-1 FITC), CD14 (anti–Leu-M3 FITC), CD15 (anti–Leu-M1 FITC), CD15 (L16 FITC), CD35 (CR I FITC), Ig isotype controls conjugated to phycoerythrin (PE) and FITC, CD33 (anti–Leu-M9 PE), CD11b (anti–Leu-15 PE), CD10 (CALLA PE), CD13 (anti–Leu-M7 PE), and CD11c (anti–Leu-M5 PE). All MoAbs were purchased from Becton Dickinson Immunocytometry

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Systems ([BDIS] San Jose, CA). FITC-conjugated polyclonal antibodies used were directed against human IgG, IgM, IgD, IgE, IgA, and κ, and the PE-conjugated polyclonal antibody was directed against human λ. These antibodies were purchased from TAGO (Burlingame, CA). The pellet of the immunofluorescent-labeled cells was resuspended in 1 mL of 1% paraformaldehyde in PBS. LDS-751 was added to the fixed cell suspension in order to exclude non-nucleated and damaged cells from the measurements by gating on fluorescence intensity typical for intact nucleated cells.[27] For cell sorting, cells were not fixed but were resuspended in RPMI 1640 containing 10% fetal calf serum (FCS).

Flow cytometry. Flow cytometric analysis was performed on a FACScan (BDIS). Data acquisition was performed with the FACScan Research Software (BDIS), whereas the analysis of the five dimensional data was performed with the PAINT-A-Gate software (BDIS).[27,28] Forward light scattering, orthogonal light scattering, and three fluorescence signals were determined for each cell and stored in listmode data files.

The cell sorting was performed on a FACStar Plus (BDIS). Cells were sorted into RPMI containing 10% FCS. The sorted cells were centrifuged for 5 minutes at 200 *g* and resuspended in 100 μL RPMI 1640 containing 10% FCS. Cytospin preparations were made with a Shandon Cyto-centrifuge (Southern Product Ltd, Astmoor, England); for each cytospin preparation 10,000 sorted cells were used. The slides of each sort were stained with Wright's stain or fixed (95% ethanol, 5% glacial acetic acid at −20°C) and stained with κ FITC and λ PE polyclonal antibodies. The slides were examined with a light or fluorescence microscope.

RESULTS

Identification of plasma cells in human bone marrow using flow cytometry. The low frequency of the plasma cells and the lack of specific cell surface antigens have been significant impediments for an accurate identification of plasma cells. These limitations could be overcome by identifying normal human plasma cells by combining two high-resolution, light-scattering parameters with the quantitative expression of the CD38 antigen on a flow cytometer. Damaged and non-nucleated cells were excluded from the measurements by gating on the cells with intermediate LDS-751 fluorescence.[27]

Figure 1 (A through C) shows three two-parameter projections correlating forward light scattering, orthogonal light scattering, and CD38 antigen expression. The orthogonal light scattering signals were transformed using a polynomial function in order to increase the resolution between the nucleated cell populations.[29] In this figure, the plasma cells were located in a unique position in the correlation of the three parameters and are black, whereas all other cells remain gray. Only six black-colored dots of 5,000 were identified as plasma cells in Fig 1 (A through C). For comparison the positions of mature neutrophils (N), monocytes (M), lymphocytes (L), and relatively mature nucleated erythroid cells (E) are indicated in the figures.[30,31]

To effectively enrich the plasma cells, the neutrophils and relative mature nucleated erythroid cells were excluded from the measurements by applying an electronic gate on the two light-scattering parameters as illustrated in Fig 1 (D through F). The apparent increase in frequency of the plasma cells is demonstrated by the increased number of black cells as compared with the gray-colored cells. The positions of immature monomyeloid (IM), immature B-lymphocytes (IB), and natural killer cells (NK) are indicated in these displays (Fig 1, D through F; defined by MoAbs and light scattering properties; data not shown).

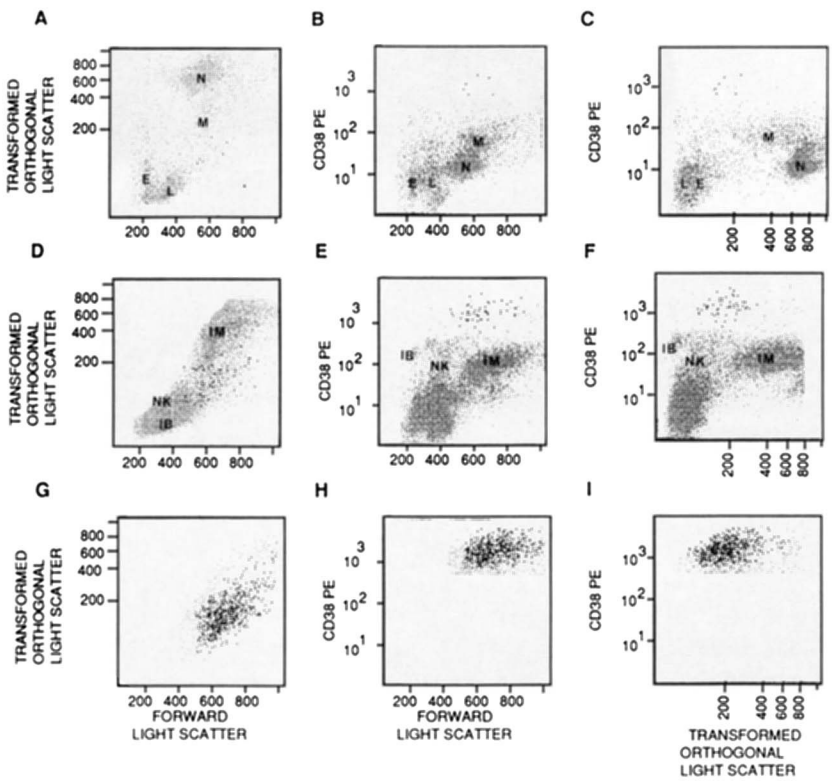

Fig 1. Identification of plasma cells. A normal bone marrow cell preparation was stained with CD38 and analyzed on a FACScan. (A through C) Three two-parameter projections of forward light scattering, transformed orthogonal light scattering, and CD38 antigen expression. The plasma cells are black, whereas all other cells remain gray. Only six black cells of 5,000 are identified as plasma cells. For comparison the positions of mature neutrophils (N), monocytes (M), lymphocytes (L), and relatively mature nucleated erythroid cells (E) are indicated. (D through F) Identical projections as shown in A through C, but applying a gate on the two light scattering parameters to increase the relative number of plasma cells. This relative increase is visualized by the increased number of black cells as compared with gray cells. Here the positions of immature monomyeloid (IM), immature B lymphocytes (IB), and natural killer cells (NK) are indicated. (G through I) Identical projections as shown in A through C with further increase of the relative number of plasma cells by exclusion of all cells not brightly expressing the CD38 antigen in addition to the light scattering gate. The plasma cells indicated with black are identified as a homogeneous cluster of cells.

A further enrichment of the relative number of plasma cells was obtained by excluding all cells not brightly expressing the CD38 antigen in combination with the light-scattering gate. The plasma cells, indicated as black dots, are identified as a homogeneous cluster of cells illustrated in Fig 1 (G through I).

Confirmation of the flow cytometric identification of plasma cells. The cells, identified as plasma cells in Fig 1, were sorted then placed on microscope slides using a cytocentrifuge and stained with Wright's stain. A typical example of the purified Wright-stained cells revealed a homogeneous population of cells with the characteristic morphology of plasma cells (Fig 2). Further evidence of the identity of these cells was obtained by intracellular staining of the sorted cells with polyclonal antibodies directed against the light chains, ie, antihuman κ FITC and antihuman λ PE. All cells stained with either κ FITC or λ PE, not both. The purity of the sorted plasma cells in four different sorts was 93%, 100%, 99%, and 99%, respectively. In the sorted cell fractions not expressing the CD38 antigen and in the cell fractions dimly expressing the CD38 antigen, no plasma cells could be found based on visual counts of 1,000.

Frequency of plasma cells in normal human bone marrow. The frequency of plasma cells in seven normal bone marrow aspirates was determined using the approach illustrated in Fig 1. The frequency of plasma cells was low, averaging 0.25% (n = 7), but relatively constant between individuals (range 0.14% to 0.30%, SD = 0.05). To examine the reproducibility of such determinations, the percentage of plasma cells was repeated in 17 separate preparations of a single bone marrow aspirate. The within-sample coefficient variation (CV) of 4% (range 0.22% to 0.26%) is remarkable for a population of such low frequency.

Identification of two populations of plasma cells. Two distinct populations of plasma cells could be observed when other MoAbs were used to further characterize the plasma cells. In the experiment illustrated in Fig 3, a normal bone marrow cell preparation was stained with CD38 PE in combination with either FITC-conjugated CD22 (Fig 3, C and D), antihuman IgE (Fig 3, E and F), HLA-DR (Fig 3, G and H), or an irrelevant antibody (Fig 3, A and B). In Fig 3A the correlation between the light scattering parameters is shown and in Fig 3B the correlation between CD38 and the FITC-conjugated irrelevant antibody is illustrated. A boundary was established using the negative control to identify the positively stained cells. Plasma cells that were unstained appear black, while those that passed this boundary are colored blue. Note the appearance of few blue-colored cells in Fig 3, A and B.

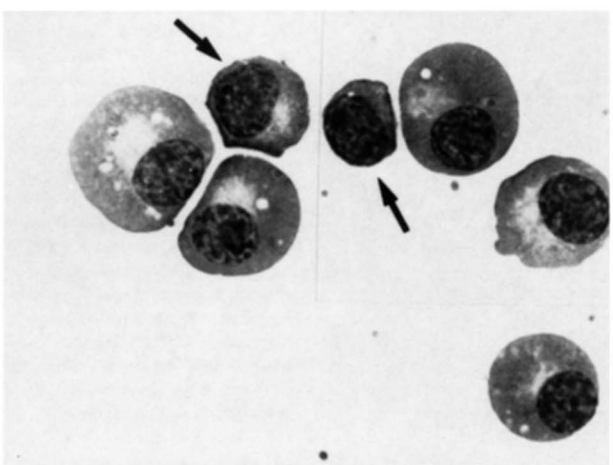

Fig 2. The cell population inferred as plasma cells in Fig 1 were sorted. An example of Wright's-stained sorted cells is illustrated here. All cells had a small, excentric nucleus, clocklike dispersed chromatin, and dark blue cytoplasm with perinuclear clearing typical for plasma cells. Two lymphoplasmacytoid cells among the plasma cells are indicated with arrows.

The expression of cell surface CD22 on plasma cells is illustrated in Fig 3, C and D. The plasma cells that did not express CD22 were depicted black whereas the plasma cells which bound CD22 are colored blue. Note that all the blue ($CD22^+$) cells are considerably smaller by forward light scattering as compared with the black ($CD22^-$) cells (Fig 3C). The frequency of the $CD22^+$ plasma cells ranged from 15% to 37% of all plasma cells (mean 25%, n = 7, Table 1).

Microscopic examination of sorted plasma cells showed that the plasma cells expressing $CD22^+$ had the typical morphologic features of lymphoplasmacytoid cells (typical examples are indicated with arrows in Fig 2), whereas the $CD22^-$ cells showed the morphology of typical mature plasma cells (Fig 2). The intracytoplasmic density of Ig light chains detected by standard fluorescence microscopy was considerably less in the $CD22^+$ populations as compared with the $CD22^-$ plasma cell population. This further confirmed the distinction between "early" lymphoplasmacytoid plasma cells and "late" mature plasma cells.

The expression of cell surface IgE on plasma cells illustrated in Fig 3, E and F was studied. The plasma cells with no detectable IgE are depicted black whereas the plasma cells bearing IgE are colored blue. Note that the same size distinction in light scattering was observed among plasma cells positive for IgE as was seen for CD22, indicating that the same population was detected. The frequency of the IgE^+ plasma cells was close to that found of the $CD22^+$ plasma cells (range 14% to 38%, mean 24%, n = 7, Table 1).

A similar size difference was observed for the plasma cells expressing $CD35^+$ as compared with $CD35^-$ (data not shown). CD23 ($Fc\epsilon_{low}$) could not be detected on either of the populations.

For a comparison, the expression of HLA-DR was studied in the same experiment, illustrated in Fig 3, G and H. The plasma cells not expressing HLA-DR are depicted black, whereas the plasma cells expressing HLA-DR are colored blue. In this case, no correlation with either the small or large plasma cell population was observed; the blue and black cells were overlapping in the correlation of both light scatter parameters.

Cell surface characterization. The presence of Ig heavy chains on the cell surface of the normal plasma cells was studied by staining bone marrow cells with CD38 PE and FITC-conjugated polyclonal antibodies directed against human IgG, IgM, IgD, IgE, and IgA. In Fig 4 the presence of cell surface IgG, IgM, and IgD on a typical normal bone

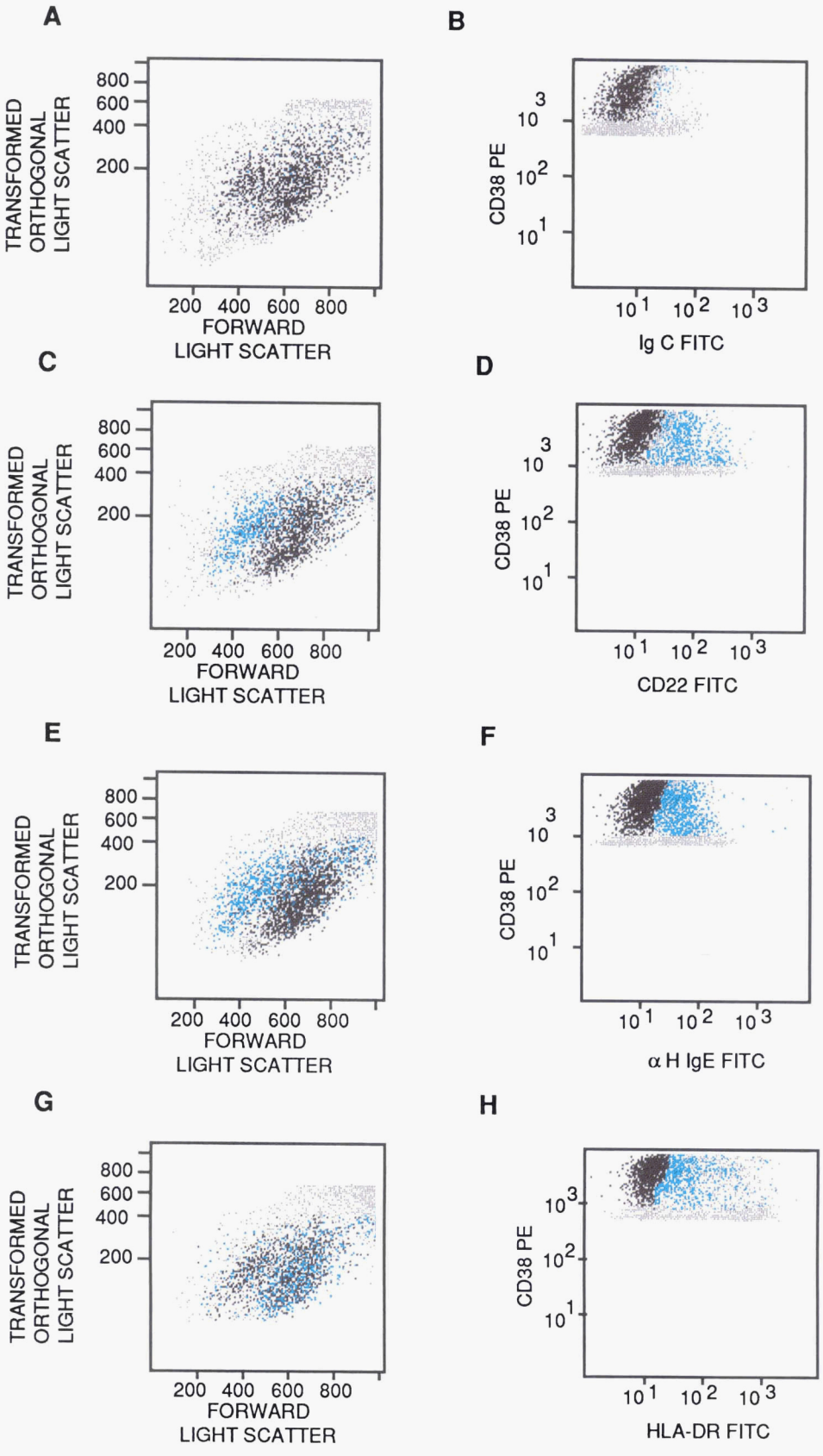

Fig 3. Two populations of normal plasma cells exhibit different phenotypes. A normal bone marrow cell preparation was stained with CD38 PE and an irrelevant FITC-conjugated antibody, CD22 FITC, human IgE FITC, and HLA-DR FITC, respectively. (A and B) The correlation of light-scattering parameters and CD38 with an FITC-conjugated irrelevant antibody. The plasma cells are depicted black. A boundary was set based on this negative control. (C and D) Expression of cell-surface CD22 by plasma cells. The plasma cells not expressing CD22 appear as black whereas the plasma cells expressing CD22 are depicted as blue. Note that all these cells were considerably smaller as assessed by forward light scattering. (E and F) Expression of cell-surface IgE by plasma cells. The plasma cells with no detectable IgE are depicted black whereas the plasma cells bearing IgE are colored blue. Note that the population discrimination based on light scattering is similar for plasma cells expressing CD22, as it is for IgE, indicating that the same population is detected. (G and H) Expression of HLA-DR by plasma cells. The plasma cells not expressing HLA-DR are depicted black whereas the plasma cells expressing HLA-DR are colored blue. In this case no correlation with either the small or large population was observed. The blue and black colored cells are mixed in the correlation with both light scatter parameters.

Table 1. Antigenic Profile of Normal Human Plasma Cells

Antibody	Mean (%)	SD	Minimum (%)	Maximum (%)
FITC conjugated Ig control	3	2.6	0	8
CD16 (Leu-11a)	5	3.2	2	9
CD19 (Leu-12)	17	11.8	3	40
CD22 (Leu-14)	25	7.5	15	37
CD20 (Leu-16)	20	26.5	4	79
HLA-DR	32	19.0	13	60
CD45 (Hle-1)	58	20.0	28	94
CD14 (Leu-M3)	6	7.3	0	17
CD15 (Leu-M1)	3	1.5	1	5
CD15 (L16)	1	1.4	0	3
CD35 (CR1)	21	1.1	2	36
PE conjugated Ig control	5	4.8	0	13
CD33 (Leu-M9)	14	11.7	3	33
CD11b (Leu-15)	41	22.1	16	73
CD10 (CALLA)	26	17.8	4	55
CD13 (Leu-M7)	16	8.4	3	25
CD11c (Leu-M5)	43	14.8	22	65

Results are percent of plasma cells expressing the antibody; results of seven samples analyzed.

marrow cell preparation is illustrated. In Fig 4A the correlative display of both light scattering parameters is shown; the neutrophils and relatively mature erythroid cells were excluded from the measurement. As a negative control, the correlative display of CD38 antigen expression and an irrelevant FITC-conjugated MoAb is shown in Fig 4B. The plasma cells are depicted black, whereas all other cells remain gray.

The presence of cell surface IgG on bone marrow cells was studied as illustrated in Fig 4, C and D. Cell surface IgG could be detected on a large proportion of the plasma cells (compare black cells in Fig 4B with those in 4D). In contrast, monocytes, colored yellow, have a different location in the correlation of both light-scattering parameters, stain with IgG FITC (presumably through Fc receptor bound IgG), and express lower levels of the CD38 antigen.[23] A subpopulation of mature B lymphocytes expressing cell surface IgG, which lack the CD38 antigen, were colored red. These cells were distinct from NK cells (IgG bound through Fcγ) because NK cells express the CD38 antigen.[26]

In the experiment illustrated in Fig 4E and F, the presence of cell surface IgM was studied. A relatively small proportion of the plasma cells had detectable surface IgM (compare Fig 4B with 4F). Early B lymphocytes are depicted blue; these cells coexpress CD38 and cell surface IgM,[22,24] as well as CD10 (data not shown). As compared with the blue–colored B lymphocytes, the red–colored, more mature B lymphocytes did not express the CD38 antigen but expressed cell surface IgM.

In the experiment illustrated in Fig 4, G and H, the presence of cell surface IgD was studied. Virtually none of the black colored plasma cells expressed cell surface IgD. The relative number of blue–colored B lymphocytes was clearly decreased as compared with the blue IgM-colored cells (Fig 4, E and F). The latter is indicative for a later expression of cell surface IgD on B lymphocytes as compared with IgM.[32] The red-colored mature B-lymphocytes are further differentiated; these cells do not express the CD38 antigen but express cell surface IgD.

The variation in expression of cell surface immunoglobulin on normal human plasma cells was determined for seven donors and illustrated in Table 2. In addition, the expression of a variety of cell surface antigens on normal human plasma cells was determined in seven normal donors (Table 1). At least 500 plasma cells were analyzed by flow cytometry for each determination of the number of plasma cells that expressed or lacked a specific antigen.

DISCUSSION

The analysis of the composition of human bone marrow aspirates is complicated by the presence of multiple cell lineages in bone marrow aspirates as well as a variety of maturational stages within each cell lineage. The introduction of multiparameter flow cytometry accelerated the studies on normal hematopoietic cell differentiation.[3,9,10,23,28,30,33-36] Normal human erythroid, megakaryocyte, monomyeloid, and early B-cell differentiation have been studied extensively in bone marrow aspirates.[3,9,10,30,31,36,37] Although normal early B-cell differentiation is well documented,[2-10,38] little is known of the terminal differentiation of the B lymphocytes into the plasma cells resident in the bone marrow.[8,13]

In this study we report the identification and characterization of the end stage of B-cell differentiation, ie, the plasma cell by high-resolution flow cytometry. The plasma cells could be uniquely identified by their location in a three-dimensional space created by forward light scattering, transformed orthogonal light scattering, and quantitative CD38 expression. The plasma cells could be identified in purities greater than 99% as analyzed by cell sorting. In the fractions depleted of the plasma cells, no plasma cells could be found, indicating that the majority of, if not all, plasma cells in normal human bone marrow aspirates could be accounted for. The high purity of the plasma cells obtained by high-resolution flow cytometry opened the opportunity to study the cell surface phenotypes of normal plasma cells.

The average frequency of the plasma cells in normal bone marrow aspirates was low: 0.25% of nucleated bone marrow cells with a remarkable small interdonor variation (SD = .05). The frequency of the plasma cells are in agreement with earlier studies enumerating plasma cells using fluorescence and light microscopy.[7,8] The small interdonor variation permits the usage of this technique to detect abnormal high frequencies of plasma cells in bone marrow aspirates.

A detailed analysis of the normal bone marrow plasma cell population demonstrated the existence of two populations of plasma cells, ie, early lymphoplasmacytoid plasma cells and late mature plasma cells. The lymphoplasmacytoid cells were distinguished from the mature plasma cells by their relatively low forward light scattering and the expression of CD22, CD35, and sIgE. The morphologic features of the lymphoplasmacytoid cells and their lower density of intracytoplasmic Ig light chains strongly suggest that these are early plasma cells. The presence of sIgE on the lymphoplasmacytoid cells was surprising and suggested the presence of low-affinity receptors for IgE, which have been shown to

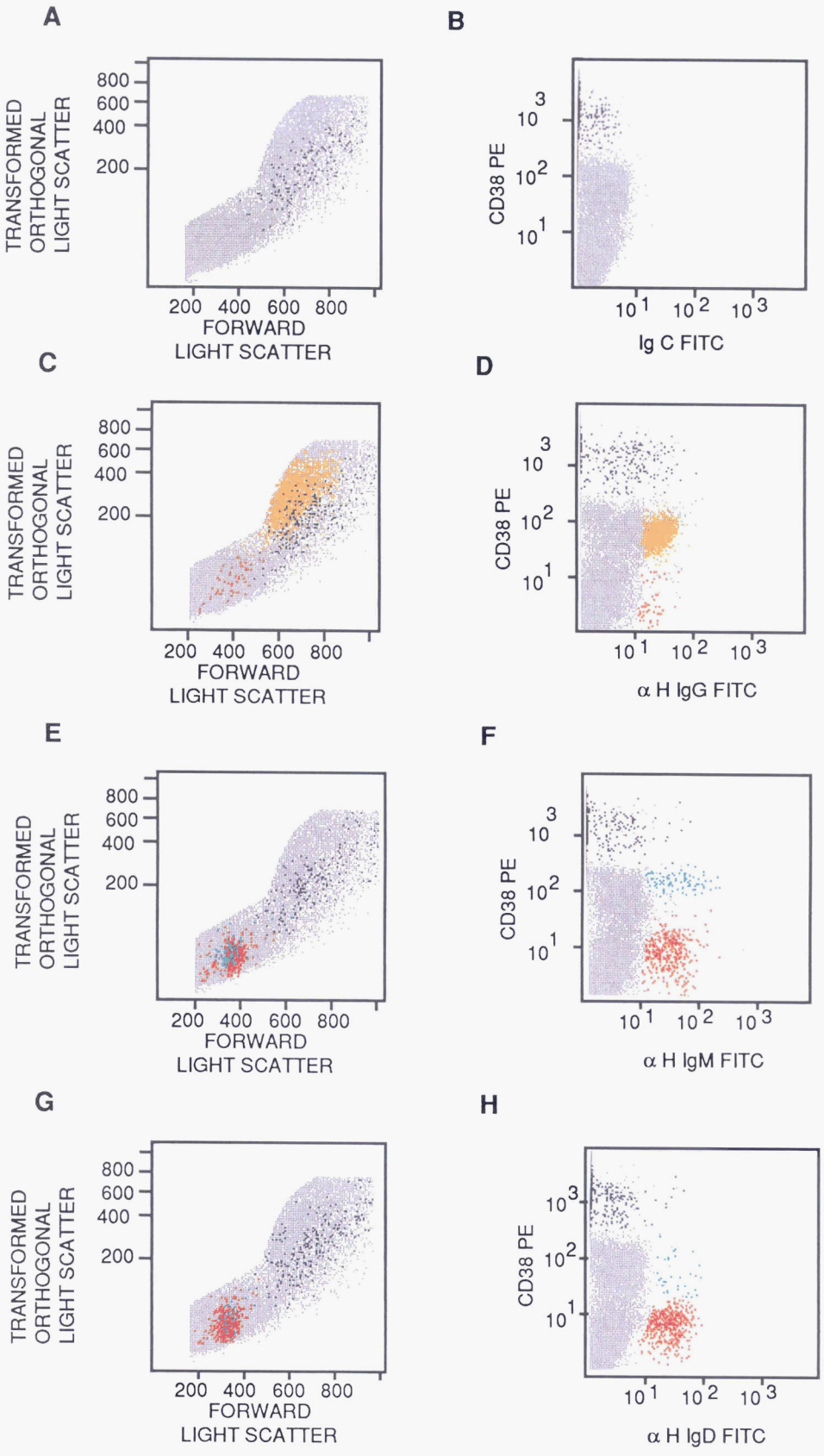

Fig 4. Cell-surface Ig expression of normal bone marrow cells. Bone marrow cells were stained with CD38 PE and an irrelevant FITC-conjugated antibody, FITC-conjugated polyclonal antibodies directed against human IgG, IgM, and IgD, respectively. (A and B) The correlated display of light-scattering parameters and display of CD38 antigen expression with an irrelevant FITC-conjugated antibody. The plasma cells are depicted black, whereas all other cells remained gray. The neutrophils and relatively mature erythroid cells were excluded by light scattering from the measurement. (C and D) The presence of cell-surface IgG on bone marrow cells. Cell-surface IgG could be detected on a large proportion of the plasma cells (compare black cells in Fig 4B with those in Fig 5D). The monocytes (colored yellow) occupied a characteristic location in the correlation of light-scattering parameters, stained with IgG FITC through Fc receptor bound IgG, and expressed the CD38 antigen. A subpopulation of mature B lymphocytes expressing cell-surface IgG and lacking CD38 are colored red. (E and F) The presence of cell-surface IgM on bone marrow cells. A relatively small proportion of the plasma cells had detectable surface IgM (compare Fig 4B with Fig 4F). Immature B lymphocytes, are colored blue, coexpressed CD38 and cell surface IgM. Mature B lymphocytes, colored red, did not express the CD38 antigen but expressed cell-surface IgM. (G and H) The presence of cell-surface IgD on bone marrow cells. Virtually none of the black plasma cells expressed cell-surface IgD. The relative number of blue-colored immature B lymphocytes is clearly decreased as compared with Fig 4, E and F. The more mature B lymphocytes that are colored red did not express the CD38 antigen but did express cell-surface IgD.

Table 2. Cell-Surface Ig Expression of Normal Human Plasma Cells

Antibody	Mean (%)	SD	Minimum (%)	Maximum (%)
Ig control	3	2.6	0	8
IgG	52	14.8	29	67
IgA	33	11.1	17	46
IgE	24	9.9	14	38
IgD	7	3.8	02	12
IgM	35	24.5	11	78

Results are percent of plasma cells expressing the antibody; results of seven samples analyzed.

appear after B-cell activation in vitro.[39] The absence of staining with anti-CD23 MoAbs on plasma cells could be a result of IgE occupying the receptor. However, it is possible that the presence of sIgE might be a result of IgE production in these plasma cells. Determination of the heavy chain production of the plasma cell populations will answer this question but has not yet been performed.

The presence of cell surface Igs was studied on the majority of normal plasma cells. No distinction was made between Fc receptor bound and transmembrane Ig. In concordance with other studies, the majority of the plasma cells expressed IgG, although significant interdonor variation was observed (Table 2).[8] The percentage of plasma cells expressing different heavy chains often exceeded 100%, indicating that more than one immunoglobulin was expressed by the plasma cells as described by MacKenzie.[32]

Although the frequency of the plasma cells in the donors studied was rather constant, the plasma cell population within and between normal donors was extremely heterogeneous with respect to the cell surface antigen expression. Only the CD15 and CD16 antigens could not be detected on normal plasma cells, whereas all other antigens studied could be found on a variable number of plasma cells. Most remarkable was the expression of myeloid antigens such as CD33 and CD13, as well as the early B-cell antigen CD10 on some plasma cells.

In acute lymphocytic leukemia (ALL) as well as acute myeloid leukemia (AML) combinations of antigens can be found on the leukemic cells that do not have a counterpart in normal human bone marrow.[40-44] The studies reporting on cell surface expression of plasma cell malignancies suggested that antigens were aberrantly expressed in myeloma in an analogous fashion to that described in ALL and AML.[13-20] The present study clearly indicates the presence of myeloid as well as early B-cell antigens on normal plasma cells, not just on the malignant counterpart. The presence of these antigens on normal plasma cells was surprising. The fact that these antigens were not found on all plasma cells and were expressed at different levels on the positive cells is different from other antigens identifying distinct maturational stages. It is possible that these myeloid and or early B lymphoid antigens on plasma cells are not functional but are products of small amounts of residual RNA present in the cells, which is amplified through transcription because of the tremendous protein synthesis occurring in the plasma cells. The phenotypic heterogeneity within the normal plasma cells limits the ability of defining myeloma cells based on aberrant expression of antigens.

In this study we have shown that during B-cell maturation the CD38 antigen is lost; ie, CD38 is present on the earliest recognizable B cells ($CD10^+$, $CD34^+$, $CD20^-$) and lost before sIgD is present on the cell surface, ($CD34^-$, $CD10^\pm$, $CD20^\pm$).[9,10] The CD38 antigen density on the plasma cells is re-expressed during terminal maturation (at levels at least tenfold higher as compared with the immature B lymphocytes). Because we have not detected cells expressing the relatively late B cell antigens CD21 and CD22, which also expressed CD38 (a phenotype expected for activated B cells), either the frequency of such activated B cells is below the detection limit (<0.01%) or activation of the B-lymphocytes occurs outside the bone marrow and the antigen-primed B cells migrate back to the bone marrow for Ig secretion. In vitro studies have shown that resting splenic B cells do not express the CD38 antigen, but after stimulation the CD38 antigen appears in synchrony with the appearance of plasma cells.[12] In view of these observations, and the studies in mice that the majority of serum IgG results from bone marrow plasma cells,[45] we favor the hypothesis that early B-cell differentiation occurs within the bone marrow, but the virgin B-lymphocytes leave the bone marrow compartment returning to the bone marrow as early plasma cells after antigen stimulation. Improvement of the knowledge of the migration patterns of normal B-lymphocytes is imperative for a better understanding of the preferential localization and migration patterns of B-cell malignancies. This study provides the means to specifically identify early and late plasma cells in normal human bone marrow, thereby opening new prospectives to explore the expression of distinct adhesion molecules (homing receptors) on the cells surface of cells along the B-cell lineage. Assessment of the tissue-specific ligands (vascular adressins) for the adhesion molecules then predict the preferential migration pattern of the identified cell population.

A major advantage of the multiparameter flow cytometric approach to assess B-cell differentiation is that one not only can identify plasma cells and their precursors but also can sort each identified population on a single-cell basis, permitting functional studies in which the behavior of normal and abnormal (myeloma) plasma cell populations can be compared.

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