Non-secretory Multiple Myeloma with Azurophilic Granules and Vacuoles: An Immunological and Ultrastructural Study

Jun KUYAMA, Satoru KOSUGI, Hironori TAKE, Tatsuo MATSUYAMA and Yoshio KANAYAMA*

Abstract

Cytoplasmic vacuolation was seen in patients with a variety of plasma cell dyscrasia. We report here a case of leukemic non-secretory multiple myeloma with many azurophilic granules. By electron microscopy, the myeloma cells were found to have a well-developed rough endoplasmic reticulum and a clear Golgi apparatus, and azurophilic granules were identified as phagocytic vacuoles. In addition to myeloma markers, the cells were positive for B cell-associated, myeloid and stem cell markers. The diagnosis is difficult because of its misleading morphology and unusual surface markers. We consider that electron microscopy is useful for the identification of cell lineage in this disorder.

Case Report

In April 2002, an 82-year-old woman who had complained of lumbago was referred to our hospital because of abnormal cells in peripheral blood. On her first admission, physical examination did not show lymphadenopathy or hepatosplenomegaly. The results of laboratory studies in serum showed a soluble interleukin-2 receptor level of 959 U/ml, a lysozyme level of 2.1 mg/l, and normal hepatic and renal functions. Quantitative assays of immunoglobulins in serum gave values all within the normal ranges. A B-J protein test was negative and immunoelectrophoresis assays in serum and condensed urine did not show any monoclonal band.

Analysis of peripheral blood indicated the presence of anemia (hemoglobin level, 8.4 g/dl), thrombocytopenia (platelet count, 61×10^9/l), and leukopenia (white blood cell count, 1.9×10^9/l; abnormal cell differential 33.5%, neutrophil differential 12.0%, monocyte differential 0%). The results of all coagulation tests were within the normal ranges. Examination of a bone marrow aspirate revealed that the abnormal cell differential was 91.6% of all the nucleated cells. The abnormal cells ranged from 15 to 30 μm in size. The nucleus was oval with fine chromatin and a prominent nucleolus. In more than half of the abnormal cells, coarse azurophilic granules filled a large part of the cytoplasmic space, except for the perinuclear clear zone. The remainder of the abnormal cells had an eccentrically-placed nucleus, basophilic cytoplasm, well-developed perinuclear clear zone, the assembly of azurophilic granules (namely inclusions) in the periphery of cytoplasm, and many vacuoles in the cytoplasm (Fig. 1). These findings appeared to be characteristic of myeloma cells except for the granules and cytoplasmic vacuoles.

The granules and inclusions of the abnormal cells were...
negative for myeloperoxidase, sudan black B and naphthol AS-D chloroacetate esterase (Fig. 2A), and were weakly positive for α-naphthyl butyrate esterase (ANBE) (Fig. 2A). ANBE reaction was inhibited by NaF (Fig. 2B). They were positive for periodic acid-Schiff reaction (Fig. 2C) and strongly positive for acid phosphatase staining (Fig. 2D).

Cytogenetic analysis of a bone marrow aspirate revealed a normal female karyotype. No major-BCR/ABL fusion gene or PML/RARα fusion gene was detected by fluorescence in situ hybridization using the bone marrow cells.

Flow cytometric (FCM) analysis performed in April 2002 during her first admission revealed that the abnormal cells obtained from a bone marrow aspirate expressed TdT (33.5%), CD10 (25.2%), CD13 (19.5%), CD19 (81.0%), CD24 (82.5%), CD33 (56.4%), CD34 (98.6%), CD38 (73.2%), and HLA-DR (90.0%) antigens. The FCM analysis showed negative for CD3 (1.0%), CD5 (1.8%), CD7 (0.9%), CD41 (0.2%), CD56 (0.6%), PCA-1 (3.0%) and GP-A (1.6%) antigens. By Southern blot hybridization analysis using a bone marrow aspirate, the heavy chain gene (JH probe) showed different bands from the controls, indicating heavy chain gene rearrangement (Fig. 3). The T cell receptor gene (Cβ probe) showed a germline configuration.

Transmission electron microscopy using the mononuclear cells of a bone marrow aspirate was performed. The abnormal cells had a heterochromatin nucleus, a prominent nucleolus, well-developed strands of rER, and a clear Golgi apparatus. These electron microscopic features were usually seen in multiple myeloma cells. In addition, almost all of the abnormal cells contained many cytoplasmic vacuoles (Fig. 4A). Each vacuole was surrounded by a limiting membrane without ribosomal adhesion. Direct continuity was not observed between the vacuoles and the Ig production-associated organelles such as rER and Golgi apparatus. Vacuolar contents were generally scanty, but membrane debris-like structures were often seen in the vacuolar lumen (Fig. 4B).

These ultrastructural findings suggested that these vacuoles might be derived from the phagocytic vesicles of lysosomal system. Except for the vacuoles, we were unable to detect any structure corresponding to the granules and inclusions in the abnormal cells. We assume that the contents of the granules and inclusions might be easily washed away in a process of glutaraldehyde fixation or might be unstainable to post-fixation by osmium-tetroxide.

Both bone X-ray survey and bone scintigraphy showed osteolytic changes and compression fractures of lower thoracic and upper lumbar vertebrae by the infiltration with abnormal cells, which brought about intractable lumbago. Based on all these findings, we diagnosed her as having leukemic multiple myeloma. Taking her age into consideration, we did not use any cytotoxic drug for myeloma and gave blood transfusions when necessary. Although her neutrophil counts continued to fall below 0.3×10^9/l for more than one year, she had not suffered from any serious infection and had been well until June 2003. In July 2003, she was readmitted to our hospital for the treatment of paralytic ileus and bacteremia by Clostridium perfringens infection. Fortunately, she showed a remarkable recovery with proper use of antibiotics.

In September 2003 during her second admission, CD38 gating FCM analysis was done using a bone marrow aspirate. The myeloma cell differential was 91.2% of all the nucleated cells. The second FCM analysis showed CD13 (6.0%), CD19 (85.9%), CD33 (54.1%), CD38 (96.9%), CD45 (7.8%), CD49d (96.8%), CD49e (84.6%), CD56 (0.9%), CD138 (3.0%), bcl-2 (74.2%), and p-53 (1.2%). The CD38 gating two-color FCM analysis of cytoplasmic light chains showed cytoplasmic κ/λ+ (0.0%) and cytoplasmic κ–/λ– (70.7%) (Fig. 5). In our FCM analysis, cytoplasmic μ, γ, or α heavy chain could not be examined due to technical difficulties. Judging from the results of FCM analysis, our final diagnosis was leukemic non-secretory type (λ light chain) multiple myeloma, fulfilling the criteria of non-secretory myeloma by the International Myeloma Working Group (8).

Discussion

Vacuolated plasma cells were observed in patients with μ-chain disease (1), γ-chain disease (2), primary macroglobulinemia (3), and other plasma cell dyscrasias (4–7). One possible mechanism regarding the vacular formation in plasma cell dyscrasia is that the abnormal immunoglobulin (Ig) produced in rER might be unable to follow a normal secretory pathway and the unsecreted portion of the Ig is then accumulated in Golgi vesicles (1, 2). Another postulated mechanism is that the abnormal Ig-containing vesicles are
engulfed by the autophagocytosis of the phagocytic vesicles, giving rise to the vacuolar formation (3–6). By the use of immuno-electron microscopy, Kanayama et al observed that in two cases of B-J myeloma and IgG myeloma the same isotypic Ig as detected in the rER was localized in the vacuoles derived from multivesicular body, which was considered to play a physiological role as a prelysosomal compartment (5). Yasuda et al reported that in two cases of macroglobulinemia and B-J myeloma the acid phosphatase activity was ultrastructurally distributed in all of the vacuoles but intracellular Ig was found only in a small portion of the vacuoles (6). Therefore, it seems probable that the lysosomal system rather than the Ig-secreting system might take a major part in the vacuolar formation of plasma cell dyscrasia. In this case, we assume that the λ light chain which was produced in rER and assembled in Golgi apparatus could not enter into normal secretory pathway by some defect in intracellular transport mechanism. This might trigger the enhancement of lysosomal activity and the formation of phagocytic vacuoles.

Normal plasma cells are heterogeneous and may not infrequently express the antigens CD9, CD10, CD13, CD20, CD33 and HLA-DR (9). This antigenic heterogeneity may reflect a spectrum of differentiation that ranges from the immature plasmablast to the mature plasma cell (10). This heterogeneity also occurs in myeloma cells which are counterpart malignant hematopoietic cells. Therefore, it is likely that myeloma cells would express the B cell-associated markers including CD10, CD19, CD20 and HLA-DR. In some instances, myeloma cells may even express the myeloid markers such as CD13 and CD33 (9). In the present case, the myeloma cells were positive not only for myeloma, B cell-associated and myeloid markers but also for the stem cell marker, CD34. Our surface marker studies imply that the

Figure 2. A) By a double esterase staining, the granules and inclusions of the myeloma cells were negative for naphthol AS-D chloroacetate esterase (NASDCAE) and weakly positive for α-naphthyl butyrate esterase (ANBE). B) ANBE reaction was inhibited by NaF. Arrows in A and B indicate NASDCAE positive neutrophils. The granules and inclusions of the myeloma cells showed positive for periodic acid-Schiff reaction (C) and strongly positive for acid phosphatase staining (D) (smear from bone marrow, ×1,000).
myeloma cells might originate from immature plasmablasts. Interestingly, a majority of the myeloma cells were positive for CD19 and negative for CD56. Some investigators asserted that the expression pattern of CD19 and CD56 might be useful for distinguishing normal plasma cells with CD19+/CD56– from myeloma cells with CD19–/CD56+ (11). It is reported, however, that in one series myeloma cells were positive for CD19 in one case out of 45 and were negative for CD56 in 11 cases out of 53 (12). Other authors reported that a high number of clonal CD19+ cells were observed in the peripheral blood of a patient with multiple myeloma (13). Therefore, we assume that the expression of CD19+/CD56– might not exclude the diagnosis of multiple myeloma.

Finally, we encountered a leukemic non-secretory type multiple myeloma with conspicuous azurophilic granules and vacuoles in the cytoplasm. By ultrastructural observations, the myeloma cells were revealed to have well-developed rER, a clear Golgi apparatus, and many phagocytic vacuoles. In general, the diagnosis of non-secretory type multiple myeloma is rather difficult. It is particularly hard to diagnose this case because of its misleading morphology and unusual surface markers. We consider that electron microscopy is useful for the identification of cell lineage and the accurate diagnosis of this disorder.

Acknowledgements: We thank Miss Chiyomi Ohtsuka, Miss Chie
Kaneshiro, and Mr. Koichi Nakagawa in the Department of Clinical Laboratory, Toyonaka Municipal Hospital, and the staff of electron microscopic research in the Department of Pathology, SRL Inc., Hachiohji for their skillful assistance.

References


