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Original Article

CD200 Expression on Plasma Cell Myeloma Cells is Associated with the Efficacies of Bortezomib, Lenalidomide and Thalidomide

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Plasma cell myeloma (PCM) is a devastating disease with a highly heterogeneous outcome, with survival ranging from a few months to longer than 10 years. Treatment of multiple myeloma has changed markedly in the past decade due to the development of new drugs such as bortezomib, lenalidomide and thalidomide, which have greatly improved the outcome of PCM. The clinical and prognostic value of immunophenotyping in PCM remains questionable. The aim of this study was to determine the diagnostic and prognostic significance of CD200 expression in newly diagnosed PCM. We retrospectively reviewed the records of 107 patients newly diagnosed with PCM at Showa University Hospital between January 2004 and September 2013. Expression of CD200 was studied by immunohistochemistry. Clinical and pathological parameters were compared between CD200-positive and CD200-negative cases. CD200-positive PCM cases had lower serum albumin (p = 0.0001) compared to those without CD200 expression. Our results showed no significant difference in median overall survival between patients with CD200-positive and CD200-negative PCM. However, there was a strong correlation between CD200 expression and serum albumin level. In the CD200-negative group, median overall survival was significantly longer in patients who received new drug treatment. These findings suggest that CD200 expression is a useful marker for evaluation of the severity of PCM and that lack of CD200 expression may improve the sensitivity of PCM to therapy with new drugs. 〔*J Clin Exp Hematop 55(3) : 121-126, 2015*〕

Keywords: CD200, plasma cell myeloma, immunohistochemistry, albumin, new drugs

INTRODUCTION

Plasma cell myeloma (PCM) is a devastating disease with a highly heterogeneous outcome. Treatment of multiple myeloma has changed in the past decade due to development of drugs such as bortezomib, lenalidomide and thalidomide,[1-4] and the prognosis of PCM has been greatly improved by these drugs. Still, survival ranges from a few months to longer than 10 years, and several staging methods using clinical and laboratory parameters have been proposed to identify patients with poor prognoses. Among these, the Durie-Salmon staging system, which includes the levels and types of monoclonal proteins, hemoglobin concentration, serum calcium level, number of bone lesions, and creatinine level, has been widely adopted as a standard staging system.[5] Serum β_2-microglobulin (Sβ2M) measurement has emerged as the single most powerful predictor of survival in several subsequent studies. The serum albumin level has also been recognized as a significant prognostic factor that improves the prognostic significance of Sβ2M measurements. In 2005, the International Staging System (ISS) was introduced as a three-stage classification using Sβ2M and serum albumin levels as a simple, powerful and reproducible system.[6]

Multi-parameter flow cytometry immunophenotyping is increasingly used for diagnostic and prognostic evaluation of hematologic malignancies including PCM,[7] using diagnostic markers such as CD56. However, the clinical and prognostic value of immunophenotyping in PCM remains questionable.[8,9] Recently, expression of CD200 has been associated with a

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poor prognosis in PCM patients.[10] CD200, which was initially described as the OX-2 tumor antigen, is a transmembrane glycoprotein that is a potential therapeutic target due to its role in immune regulation and tolerance. CD200 is expressed on the cell surface of thymocytes, B and T lymphocytes, neurons, kidney glomeruli, tonsil follicles, syncytiotrophoblasts and endothelial cells,[11] and in melanoma, renal cell carcinoma, and ovarian tumor cells. CD200 is also expressed in hematological malignancies such as acute leukemia, chronic lymphocytic leukemia/small lymphocytic lymphoma, hairy cell leukemia, classical Hodgkin lymphoma, and angioimmunoblastic T-cell lymphoma.[12-14] It has recently been hypothesized that CD200 may play an important role in tumor progression because of its immunosuppressive effect on the host immune system.[15]

Normal plasma cells have no or weak CD200 expression in assessment by flow cytometry,[16] whereas most PCM cells express CD200 strongly. Immunophenotypic analysis is mainly used for detection of PCM cells in atypical cases of PCM. There is a paucity of literature on CD200 in PCM, and the effects of CD200 expression are unclear. Thus, CD200-negative PCM has been suggested to have better event-free survival (EFS) compared with CD200-positive PCM;[10] but loss of CD200 expression in PCM has also been associated with a more clinically aggressive disease.[17] Differences in findings may arise due to analysis of PCM cells after anti-myeloma therapy or at initial diagnosis of PCM. Regardless, it is clear that PCM may be a useful prognostic indicator. Therefore, the aim of this study was to analyze expression of CD200 in newly diagnosed PCM and to define the relationships between CD200 expression and other clinical and pathological parameters.

MATERIALS AND METHODS

The subjects were 107 patients (64 males and 43 females) of median age 69 years (range, 39-92 years). All cases were newly diagnosed with PCM at Showa University Hospital between January 2004 and September 2013. Medical records were reviewed to obtain clinical information, including therapy regimens, laboratory data, and overall survival (OS). Morphologic and immunophenotypic data were reviewed to confirm the diagnosis according to 2008 WHO criteria.

Morphological findings were obtained using H&E stains of 3-μm sections. Formalin-fixed, paraffin-embedded specimens were used for immunohistochemistry with the following antibodies: CD200 (polyclonal, goat; R&D Systems, Minneapolis, MN, USA), CD138 (clone MI15; Dako Cytomation A/S, Glostrup, Denmark), CD56 (clone 1B6; Novocastra, Newcastle, UK). CD200 and CD56 immunostained slides were defined as positive if > 30% of plasma cells had moderate to strong membranous staining. Statistical analysis was performed using JMP 11 (SAS Institute Inc., Cary, NC, USA). A χ^2 test was used to compare clinical and pathological features between the CD200-positive and CD200-negative groups. A Wilcoxon signed-rank test was used to compare laboratory data between these groups. OS was analyzed using the Kaplan-Meier method and compared by generalized Wilcoxon test. A p value < 0.05 was considered significant in all analyses.

RESULTS

The study cohort included 64 men and 43 women, with a median age of 69 years (range, 39-92 years). The immunoglobulin heavy chain type was IgG in 53 patients, IgA in 29, IgD in 1, and BJP in 30. One patient had non-secretory PCM. In the Durie-Salmon classification, 68 (64%) of patients were

Table 1. Baseline clinical and laboratory features in patients with plasma cell myeloma (n = 107)

Clinical data	No. of cases (%)
Sex	
Male	64 (59)
Female	43 (40)
Age	
Median	69 years
Range	39-92 years
Durie-Salmon stage	
I	17 (16)
II	21 (19)
III	68 (63.5)
Unknown	1 (1)
International staging system	
I	19 (18)
II	32 (30)
III	47 (44)
Unknown	9 (8)
Immunoglobulin heavy chain type	
IgG	53 (50)
IgA	29 (27)
IgD	1 (1)
Bence-Jones protein	30 (28)
Non secretory	1 (1)
Treated with new drugs	52 (49)
Bortezomib	44 (41)
Lenalidomide	24 (22)
Thalidomide	6 (6)
No-treatment with new drugs	38 (36)
Serum β_2-microgrobulin, mg/L	4.9 ± 6.3
Creatinine, mg/dL	0.97 ± 2.94
Calcium, mg/dL	9.3 ± 1.31
Albumin, mg/dL	3.3 ± 0.65
Lactate dehydrogenase, IU/L	180.5 ± 185.6
Hemoglobin, g/dL	9.9 ± 2.61

Data are shown as median ± SD.

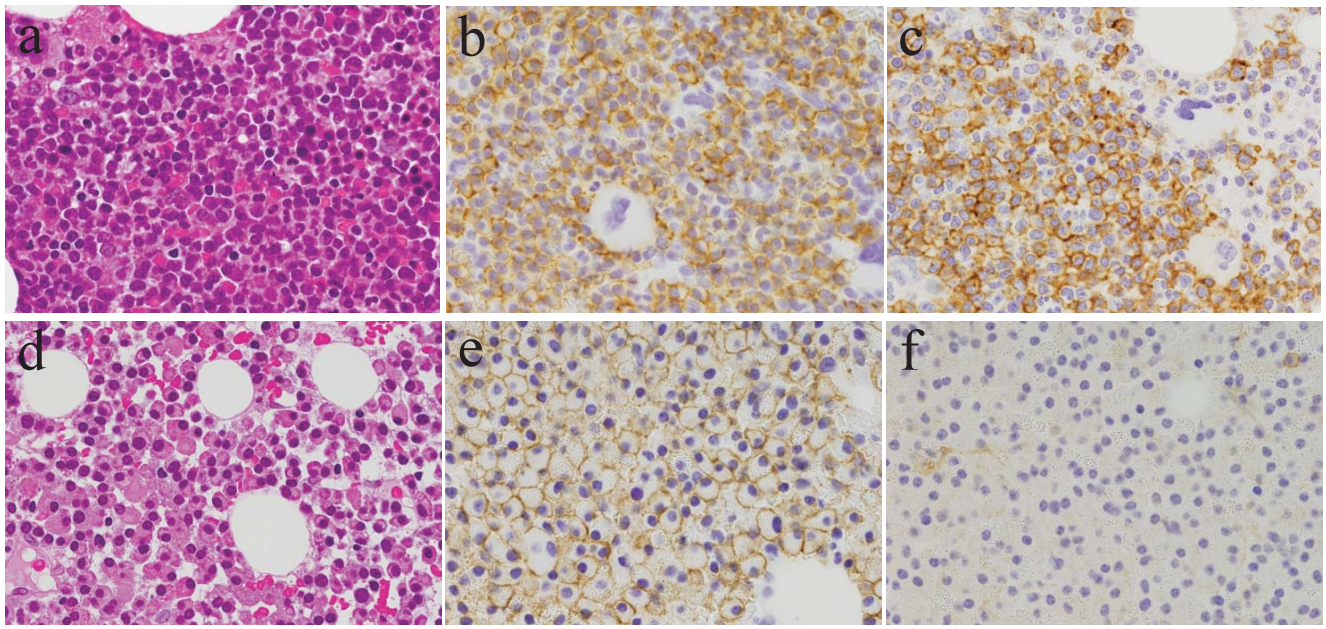

Fig. 1. CD200 expression in plasma cell myeloma. (*Upper row*) Representative case with stable CD200 expression: (*1a*) H&E stain, (*1b*) CD138 immunostain and (*1c*) CD200 immunostain. (*Lower row*) Representative case with a lack of CD200 expression: (*1d*) H&E stain, (*1e*) CD138 immunostain and (*1f*) CD200 immunostain.

in Stage III. In the ISS, 19 (18%), 32 (30%), and 47 (44%) of patients were in stages I, II, and III, respectively.[6] Among the 90 patients who received any therapy, 52 were treated with new drugs [bortezomib (n = 44), lenalidomide (n = 24) or thalidomide (n = 6)] during the course of the disease, and 38 received conventional high dose chemotherapy only. Median Sβ2M was 4.9 g/dL, creatinine 0.97 mg/dL, calcium 9.3 mg/dL, albumin 3.3 g/dL, lactate dehydrogenase 180.5 IU/L, and hemoglobin 9.9 g/dL. Baseline characteristics are shown in Table 1.

Of the 107 cases, 71 (66.3%) had CD200 expression in PCM cells (Fig. 1). The clinical and pathological features of the CD200-positive and CD200-negative groups were similar for age, gender, light chain restriction, Sβ2M level, lactate dehydrogenase, and hemoglobin (Table 2). However, significantly more patients in the CD200-positive group had a lower serum albumin (≤ 3.5 mg/dL) (71.8% vs. 38.9%, $p < 0.0001$). There was no significant correlation between CD200 expression and Sβ2M. Similar trends were seen in comparisons among ISS stages. In the CD200-positive group, the mean serum albumin level was significantly lower ($p = 0.0003$) and the rate of CD56 expression was significantly higher ($p < 0.001$) compared to the CD200-negative group ($p < 0.001$). Median OS in the CD200-positive group showed a tendency to be longer than that in the CD200-negative group, but the difference was not significant ($p = 0.31$) (Fig. 2A). The serum albumin level also had no significant effect on median OS ($p = 0.87$) (Fig. 2B).

Table 2. Comparison of clinical features in the CD200-positive and CD200-negative groups

Clinical data	CD200 (+)	CD200 (-)	*p*-value
Case number	71	36	
Age > 65 years (%)	71.8	66.7	0.650
Male (%)	60.6	58.3	0.830
κ (%)	57.8	41.7	0.150
λ (%)	38.0	52.8	0.146
IgG (%)	54.9	38.9	0.110
IgA (%)	28.2	25.0	0.726
Sβ2M < 3.5 mg/dL (%)	11.3	22.2	0.377
Sβ2M > 5.5 mg/dL (%)	11.3	47.2	0.507
Albumin < 3.5 g/dL (%)	71.8	38.9	0.001*
Hemoglobin < 10 g/dL (%)	49.3	52.8	0.848
Creatinine > 1.5 mg/dL (%)	25.5	20.0	0.780
Calcium > 10.5 mg/dL (%)	11.3	22.2	0.157
CD56 (+) (%)	81.7	52.8	0.003*

Sβ2M, serum β_2-microgrobulin; *, $p < 0.05$

In a subgroup analysis based on therapy regimens, in the CD200-negative group the median OS of patients who received new drug therapy was significantly longer than that of patients who received only conventional high dose therapy. This effect was not present in the CD200-positive group (Fig. 3). For CD56 expression, there was no significant difference in median OS in subgroup analysis based on the therapy regimen.

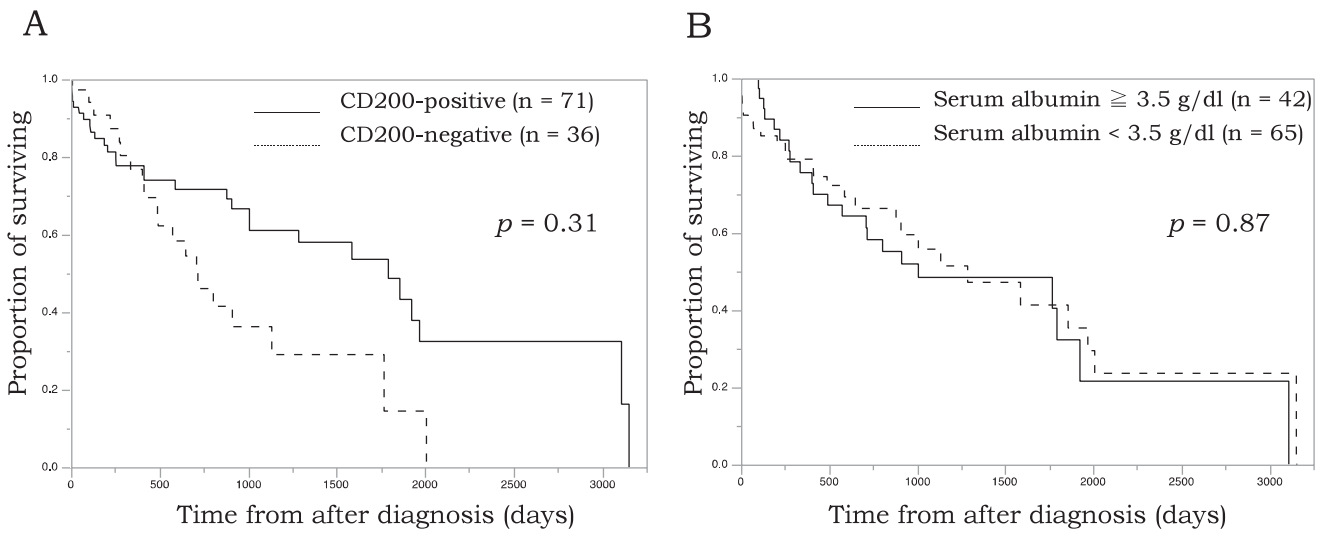

Fig. 2. Comparison of overall survival (OS) between CD200-positive and -negative cases and levels of serum albumin. (***2A***) OS in CD200-positive (n = 71) and CD200-negative (n = 36) patients with plasma cell myeloma. (***2B***) OS in patients with plasma cell myeloma with higher (< 3.5 g/dL, n = 42) and lower (< 3.5 g/dL, n = 65) levels of serum albumin.

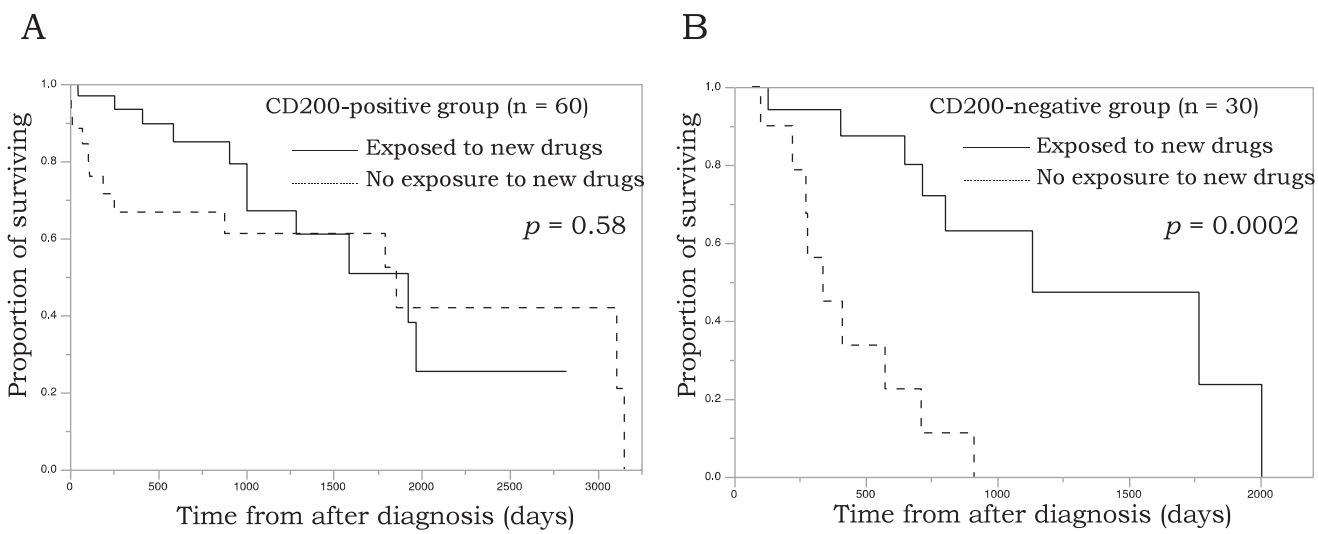

Fig. 3. Comparison of overall survival (OS) between cases with and without exposure to new drugs. (***3A***) OS in CD200-positive patients who received new drug therapy (n = 33) or conventional high dose therapy only (n = 27). (***3B***) OS in CD200-negative patients who received new drug therapy (n = 19) or conventional high dose therapy only (n = 11).

DISCUSSION

PCM is an incurable neoplastic plasma cell disorder characterized by proliferation of clonal malignant plasma cells in bone marrow. Survival rates in patients with PCM are variable, reflecting the heterogeneity of PCM cell biology. CD200 may be a useful prognostic marker in PCM because of its immunosuppressive effect on the host immune system.[10,18] Lower serum albumin is currently viewed as the most useful marker of a poor prognosis in PCM.[6,19,20]

In this study, CD200 expression was examined in 107 cases of PCM using immunohistochemistry. CD200 was expressed on PCM cells in 71% of cases, similarly to previous findings.[18] Lower Sβ2M has been found in CD200-positive cases ($p = 0.03$),[21] but our results showed no correlation of CD200 expression with low Sβ2M levels. In contrast, lower serum albumin levels were significantly more common in CD200-positive cases than in CD200-negative cases (p =

0.0001). The Sβ2M level is a powerful predictor of survival in PCM. However it should be noted that the serum albumin level improves the prognostic significance of Sβ2M, as used in the ISS. The specific cause of decreased serum albumin in PCM patients with a poor prognosis is uncertain.[6] The balance among production by the liver and degradation determines the concentration of serum albumin, and effects on the liver of interleukin-6 produced by the microenvironment of PCM cells may decrease albumin production.[19,22,23] Renal failure or nephrotic syndrome induced by PCM may also decrease the level of albumin. However, there was no significant difference in kidney function decline between the CD200-positive and -negative groups in this study, based on the levels of serum creatinine and Sβ2M.

CD200 expression was not associated with survival in our cohort, whereas a previous report found that CD200 expression is associated with worse EFS.[10,18] However, this is uncertain, because a flow cytometry study suggested that CD200-positive PCM had a better outcome than CD200-negative cases.[17] The CD200-CD200R interaction is a potential target for immunomodulation and CD200 delivers an inhibitory signal for the macrophage lineage in diverse tissues.[24] In addition to being expressed in normal tissues, CD200 is functionally involved in an immunosuppressive signaling pathway via interaction with its receptor, CD200R, with downstream effects on macrophage inhibition, induction of regulatory T cells, and inhibition of tumor-specific T cells. Molecular signaling pathways activated in the tumor environment help to create an immunosuppressive network, through which the cancer escapes detection. This could explain the worse EFS of CD200-positive PCM found in some studies.

In our study, no results seemed to depend on the CD200-CD200R interaction, and there was no difference in OS between the CD200-positive and -negative groups. This suggests that the function of CD200 and its regulation in PCM is complex, and a further study is required. As reported previously, CD200 was expressed in most cases of PCM and there was a strong correlation between expression of CD200 and CD56. CD56 is a membrane glycoprotein and adhesion molecule that belongs to the immunoglobulin superfamily. CD56 expression correlates with the ability of cells to migrate out of bone marrow, and modulation of CD56 expression is a potential mechanism of regulation of plasma cell homing to extramedullary sites.[25] Currently, CD56 is used as a diagnostic marker for PCM cells, although there is no consensus on CD56 as a prognostic marker in PCM. CD56 may be more useful than CD200 as a diagnostic marker because it is typically negative in normal plasma cells, while CD200 is weakly positive.[9] However, CD200 expression in PCM may be useful in follow up analysis due to its stability.[17,26] Furthermore, our results suggest that CD200 expression may be a good indicator for the choice of therapy.

In summary, we found that expression of CD200 is a reliable marker for PCM diagnosis. The strong correlation of CD200 expression with a low serum albumin level suggests that CD200 could also be a useful marker for evaluation of the severity of PCM. With regard to prognosis, patients who are CD200-negative may be more responsive to treatment with drugs such as bortezomib, lenalidomide and thalidomide. Thus, CD200 immunohistochemistry may be a useful indicator for the choice of PCM treatment. A further study in a larger number of cases is needed to confirm these findings.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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