Expression of CD66a in Multiple Myeloma

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CD66a is a member of the carcinoembryonic antigen family and has been suggested to function as an intercellular adhesion molecule and cell growth regulator. Expression of CD66a in myeloma cells was examined with mAb TS135 against CD66a transfectants of murine-transformed fibroblasts. The reactivity of mAb TS135 with CD66a, CD66c, and CD66e was revealed. CD66a in myeloma cells was considered to be detectable with this mAb, since CD66c and CD66e are not expressed in them. CD66a was detected in three myeloma cell lines and an IgM-producing B-cell line. In clinical bone marrow specimens, including 18 multiple myeloma, two primary macroglobulinemia, and a case of CLL-like chronic

lymphoproliferation with monoclonal IgG production, CD66a and three conventional myeloma cell markers (PCA-1, CD38, and CD56) were examined by indirect immunofluorescence assay. The results showed that 18 out of 21 cases (86%) were CD66a⁺, and PCA-1 showed the highest correlation with CD66a among conventional markers. Primary macroglobulinemia and chronic lymphoproliferation were also CD66a⁺. Two-dimensional flow cytometry with mAbs TS135 and CD38 confirmed the reactivity of TS135 with myeloma cells in those bone marrow specimens. The findings suggest that CD66a is expressed in multiple myeloma with high frequency. *J. Clin. Lab. Anal.* 16:79–85, 2002. © 2002 Wiley-Liss, Inc.

Key words: CD66a; multiple myeloma; CEA family; PCA-1; CD38

INTRODUCTION

CD66a (biliary glycoprotein (BGP)) is a member of the carcinoembryonic antigen (CEA) gene family (1), and was recently designated as CEACAM1 (2). Eleven splice variants have been revealed (2), but the largest one (BGPa) is a major transcript that encodes a type I transmembrane protein consisting of an extracellular domain with four immunoglobulin-like domains and the cytoplasmic domain. Although the *in vivo* functions of CD66a remain unknown, it has been postulated that it may act as an intercellular adhesion molecule (3), a receptor for microorganisms (4), and a growth suppressor (5,6). CD66a was originally found in the bile (7), but a subsequent immunohistochemical study disclosed a wide tissue distribution (8). With regard to hematopoietic tissues, its expression has been revealed in granulocytes (9,10), lymphocytic tumor cells (11), activated T lymphocytes and NK cells (12,13), and intestinal intraepithelial lymphocytes (14), suggesting that CD66a plays an important role in normal and transformed lymphocyte functions.

Mayne et al. (15) previously reported the reactivity of monoclonal antibody (mAb) By114 against CEA (CD66e), and non-specific cross-reacting antigen (NCA)-50/90 (CD66c) and mAb CLB-gran/10 against CEA, NCA, and BGP with cell lines and

clinical tissue specimens of hematopoietic tumors. Neither By114 nor CLB-gran/10 reacted with the lymphoid and myeloma cell lines tested. On the other hand, it is of interest that clinical tissue specimens of B-cell lymphoma, myeloma, and macroglobulinemia were positive for CLB-gran/10, whereas all of the tumor specimens tested were negative for By114, suggesting the expression of BGP in mature B-cell tumors. In addition, Hanenberg et al. (11) demonstrated the expression of CD66a/BGP in four mature B-cell lines. However, no detailed studies on myeloma have been performed to date.

We previously established a murine mAb against CD66a (BGPa) cDNA-transfected EJ-NIH3T3 cells (designated as TS135) that was shown to be weakly cross-reactive with crude CD66c extracted from transfectants or peripheral blood granulocytes with perchloric acid (16). Using this mAb, we examined the expression of CD66a in cell lines and bone marrow cells from patients with multiple myeloma.

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MATERIALS AND METHODS

Cell Lines and Human Bone Marrow Specimens

Cultured cells were maintained in RPMI 1640 supplemented with 25 mM HEPES, 25 mM sodium bicarbonate, 200 μ g/L ampicillin, 100 μ g/L kanamycin, and 10% (v/v) FCS. RPMI8226, KR12, and TAPC are myeloma cell lines, RPMI1788 is an IgM-producing B-cell line, and CHC-Y1 is a colon cancer cell line (17). The transfectants of CHO cells expressing recombinant CD66 proteins, including CD66a (BGP), CD66b (CGM6), CD66c (NCA50/90), and CD66e (CEA), were described previously (18). Human bone marrow specimens were obtained from patients in the Sapporo Medical University Hospital and were subjected to analysis of surface markers after informed consent was given.

Flow Cytometry

Indirect immunofluorescence assay was performed as previously described (19). Briefly, cells were incubated with a saturating amount of mouse mAb for 30 min on ice, washed twice with cold phosphate-buffered saline (PBS), and then incubated with FITC-conjugated rabbit anti-mouse immunoglobulin (Ig) (Dakopatts, Glostrup, Denmark) for 30 min on ice. After they were washed twice with cold PBS, cells were analyzed by flow cytometry (Epics Profile II, Coulter Electronics, FL). For bone marrow cell analysis, after red blood

cell removal under low osmotic pressure conditions, mononuclear cells were selected with the gate and analyzed. For two-dimensional flow cytometry, after the indirect immunofluorescence reaction for antigen TS135 with mAb TS135 and FITC-labeled rabbit anti-mouse Ig as described above, cells were incubated with PE-conjugated anti-CD38 mAb for 30 min on ice. They were then washed twice with cold PBS and analyzed by flow cytometry.

In addition to mAb TS135, mAbs F34-187 reacting with BGP (CD66a), NCA (CD66c), CEA (CD66e), CGM1 (CD66d), and anti-CGM6 (CD66b) 80H3 (10) were used for indirect immunofluorescence assay as first antibodies. MAS E7 against α -fetoprotein and MOPC21 were developed in our laboratory (unpublished data) and purchased from Organon Teknika (Turnhout, Belgium), respectively, and used as negative control antibodies.

Enzyme-Linked Immunosorbent Assay

An enzyme-linked immunosorbent assay (ELISA) was performed as previously described (20). MAbs TS135, anti-NCA (CD66c) F106-88, and anti-CEA (CD66e) F11-39 (10,21) were used for the ELISA.

Fig. 1. Flow cytometric analysis of the reactivity of mAb TS135 with transfectants expressing CD66a (BGP), CD66b (CGM6), CD66c (NCA), or CD66e (CEA). MAbs F34-187 and 80H3 recognize CD66acde and CD66b, respectively. MAb MOPC21 was used as a negative control antibody.

Fig. 2. ELISA of the reactivity of mAb TS135 with purified CD66c (NCA) or CD66e (CEA). MAbs F106-88 and F11-39 are specific for CD66c and CD66e, respectively.

Fig. 3. Flow cytometric analysis of the reactivity of mAb TS135 with three myeloma cell lines (RPMI8226, KR12, and TAPC) and an IgM-producing B-cell line (RPMI1788). CD38 and PCA-1 are representative con-

ventional myeloma cell markers. Anti-alpha-fetoprotein mAb E7 was used as a negative control antibody.

Statistical Analysis

The relation of the percentage of CD66a⁺ cells with that of PCA-1⁺, CD38⁺, and CD56⁺ cells was evaluated by Pearson's correlation coefficient.

RESULTS

Specificity of mAb TS135

To ascertain the specificity of mAb TS135, its reactivity with CHO transfectants expressing CD66a (BGP), CD66b (CGM6), CD66c (NCA50/90), and CD66e (CEA), and with purified CD66c and CD66e were examined. As shown in Fig. 1, mAb TS135 reacted with CD66a and CD66c, and CD66e was expressed on CHO cells. F34-187 and 80H3

were used as positive control antibodies, the latter being specific for CD66b. The reactivity of mAb TS135 with purified CD66c and CD66e was also confirmed by ELISA (Fig. 2). These data suggest that mAb TS135 probably belongs to a group of mAbs recognizing CD66acde, in agreement with findings presented at the 6th International Workshop and Conference on Human Leukocyte Differentiation Antigens (22,23).

Reactivity of mAb TS135 With Mature B-Cell Lines

As shown in Fig. 3, mAb TS135 reacted with three myeloma cell lines (RPMI8226, KR12, and TAPC) and an IgM-producing B-cell line (RPMI1788). CD38 and PCA-1, which are representative myeloma cell markers, were expressed on

Fig. 4. Flow cytometric analysis of the reactivity of anti-CD66c (NCA) mAb F106-88 with three myeloma cell lines (RPMI8226, KR12, and TAPC)

and an IgM-producing B-cell line (RPMI1788). A colon cancer cell line (CHCY-1) was used as positive control cells.

TABLE 1. Detection of TS135 (CD66a) and myeloma cell makers in bone marrow cells from patients with multiple myeloma by flow cytometry

Patient no.	Sex	Age	Diagnosis	TS135	PCA1	CD38	CD56	CD19
1	M	65	MM(IgA κ)	78.9	88	92.1	87.2	2.3
2	F	48	MM(IgA λ)	57.1	34.6	n.t.	n.t.	11.1
3	F	52	MM(IgG λ)	20.2	85.2	n.t.	n.t.	5.1
4	M	64	MM(IgG λ)	3.5	2.9	22.3	10.3	2.2
5	F	71	MM(IgG λ)	4.4	5.5	23	15.2	19
6	F	61	MM(IgA κ)	22.9	30.4	n.t.	24.5	15.2
7	F	67	MM(IgG κ)	11.6	12.6	37.2	14.1	19.4
8	M	76	MM(IgA κ)	4.4	2.8	16.7	20.9	n.t.
9	F	61	MM(IgG λ) + amyloidosis	29.2	37.4	36.8	18.5	20.2
10	M	55	MM(BJ κ) + amyloidosis	38.7	42.4	53.7	19.1	3.8
11	F	51	MM(IgG κ)	84.9	88.1	90.5	90	1.2
12	F	74	MM(IgG λ)	30.3	25.1	51.4	46.1	0.2
13	F	72	MM(IgG κ)	62.1	79.5	95.2	86.1	0.6
14	M	68	MM(BJ κ)	30.9	36.2	81.1	37.6	3.2
15	F	69	MM(IgA κ)	23.6	9.3	80.2	65.4	1.9
16	M	68	MM(IgG κ)	19.2	21.5	43	31.1	3.2
17	F	63	MM(IgG λ)	59.6	64.4	85.2	10.7	2.5
18	M	74	MM(IgG κ)	13.6	35.6	84	n.t.	1.2
19	F	78	CLL-like(IgG κ) ^a	41.2	58.6	15.6	n.t.	24.7
20	F	60	Macroglobulinemia (IgM κ) ^b	38.1	43.1	43.3	11.4	29.5
21	F	61	Macroglobulinemia (IgM κ) ^b	46	73.5	11.3	6	47.2

^aPrimary macroglobulinemia.^bChronic lymphoproliferation with monoclonal IgG secretion.

three and four out of four cell lines tested, respectively. CD66c (NCA50/90) was not detected in those cells (Fig. 4). The data suggest that CD66a is expressed on myeloma cells.

Expression of CD66a in Bone Marrow Cells From Patients With Multiple Myeloma or Macroglobulinemia

Bone marrow cells from 21 patients with multiple myeloma, primary macroglobulinemia, or chronic lymphoproliferation were evaluated by indirect immunofluorescence assay. The age, sex, and diagnosis of the patients are shown in Table 1. The specimens with the percentage of positively stained cells being more than 10% were judged to be positive for each mAb. However, it was difficult to determine the percentage of myeloma cells by this method when it was less than 10% because of recognizable background levels due to nonspecific binding of the second antibody (data not shown). Thus, 18 out of 21 (86%) specimens were considered to be positive for mAb TS135 (CD66a⁺). The percentage of CD66a⁺ cells were then compared to that of conventional myeloma cell markers (PCA-1, CD38, and CD56) and that of a pan-B-cell marker (CD19). Among them, PCA-1 showed the highest correlation with CD66a (Fig. 5). Two cases of primary macroglobulinemia and a case of chronic lymphoproliferation were also revealed to be CD66a⁺ (Table 1).

To confirm the reactivity of mAb TS135 with myeloma cells in the bone marrow, two-dimensional flow cytometry with mAbs TS135 and CD38 was performed. Four representative cases are shown in Fig. 6. Cases 6, 17, and 18 were

multiple myeloma, showing highly positive for CD38 (which is characteristic for myeloma cells (24)), with a moderate expression of CD66a. Case 19 was chronic lymphoproliferation with t(11; 14) (q13; q32) producing IgG1 M-protein, as previously described (25), which demonstrated a lower expression level of CD38 compared to multiple myeloma.

Indirect immunofluorescence assay for bone marrow cells is not highly quantitative, but is of practical use for monitoring disease activity. A time course of CD66a⁺ cells in the bone marrow specimen of case 1 (Table 1), which was re-

Fig. 5. The relationship between the percentage of cells positive for mAb TS135 (% of CD66a⁺ cells) and percentage of PCA-1⁺ cells. A significant correlation between them was observed ($r = 0.801$, $P < 0.0001$).

Fig. 6. Two-dimensional flow cytometric analysis of bone marrow cells with mAbs CD38 and TS135. Cases 6, 17, and 18 were multiple myeloma, and case 19 was chronic lymphoproliferation with monoclonal IgG production.

fractory to chemotherapy, is shown in Fig. 7. The lowest percentage of myeloma cells morphologically identified in the bone marrow was 40% during this period. The percentage of CD66a⁺ cells was shown to change almost in parallel with that of PCA-1⁺ cells and myeloma cells.

DISCUSSION

It was demonstrated in this study that three myeloma cell lines and one mature IgM-producing B-cell line reacted with

mAb TS135 recognizing CD66a, CD66c, and CD66e, but not with anti-CD66c mAb F106-88. Although the reactivity of mAb TS135 with CD66d was not tested, CD66d is known to be expressed exclusively in the granulocyte lineage (26). CD66e (CEA) is a representative tumor marker for epithelial malignancy and is not expressed in hematopoietic cells (27). For this reason, bone marrow metastasis of breast cancer cells has been detected by reverse-transcription polymerase chain reaction (RT-PCR) for CEA mRNA (28,29). This strongly suggests that mAb TS135 recognizes CD66a on these four cell lines, which made it possible for us to examine the expression of CD66a in the bone marrow cells from patients with multiple myeloma.

The percentage of CD66a⁺ cells obtained by indirect immunofluorescence assay was highly correlated with that of PCA-1⁺ cells in 21 bone marrow specimens from patients with multiple myeloma, primary macroglobulinemia, and chronic lymphoproliferation. PCA-1 is a conventional cell surface marker for myeloma cells and is also expressed in primary macroglobulinemia (30), mast cells (31), granulocytes, and monocytes (32) (although information at the molecular level is lacking). CD66a is also expressed in granulocytes and monocytes (8–10,33). In this study, a mononuclear cell fraction was selected from bone marrow cells with the gate before analysis in flow cytometry. This suggests that monocytes may have been contaminated in both the CD66a⁺ and PCA-1⁺ cell populations, causing a significant correlation between them. However, the expression level of CD38 was high in two-dimensional flow cytometric analysis with mAbs TS135 and CD38 (Fig. 6), a finding that is characteristic for myeloma cells (24). Furthermore, considering that normal hematopoiesis is suppressed in the bone marrow of patients with multiple myeloma, it is not plausible that contamination of monocytes significantly affects the percentage of CD66a⁺ or PCA-1⁺ cells.

Fig. 7. A time course of the percentage of myeloma cells TS135⁺ (CD66a⁺) and PCA-1⁺ cells in the bone marrow specimen from a patient with multiple myeloma (IgAκ) together with serum IgA level. I, MI, EI, V, P, and C indicate interferon-α (IFN-α), MP (melfar and prednisolone) + IFN-α, VP16

+ IFN-α, VAP (vincristine, adriamycin, and methylprednisolone), prednisolone, and CHOP (cyclophosphamide, adriamycin, vincristine, and prednisolone), respectively.

In addition to myeloma cells, CD66a was shown to be expressed together with PCA-1 in primary macroglobulinemia and a mature B-cell tumor with production of monoclonal IgG. The latter tumor cells were morphologically similar to chronic lymphocytic leukemia, with expression of cell surface IgG and overexpression of cyclin D1 (25). Compared to typical myeloma cells, the expression level of CD19 was increased in this case, suggesting that the maturation level of tumor cells may be between those of myeloma and macroglobulinemia. These data suggest the expression of CD66a in mature B-cell tumors, in addition to myeloma, and support the findings in previous reports (11,15).

In previous studies it was also shown that CD66a is overexpressed in lung and gastric cancers (34,35), although the mechanism for this remains unknown. On the other hand, it has been revealed to be down-regulated in various cancers of the liver (16,36), prostate (37), colon (38,39), breast (40), bladder (41), and uterus (42). Moreover, cDNA transfection of mouse and rat CD66a homologues into cultured tumor cells inhibited in vitro and in vivo tumor growth (5,6), suggesting that CD66a may function as a tumor suppressor. Further studies will be required to clarify the functional role of CD66a in myeloma cells.

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