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Expression of an Immunotherapeutic Target Antigen 17-1A for Colorectal Cancer in Multiple Myeloma

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Abstract A colorectal cancer-associated 17-1A antigen is an effective therapeutic target with monoclonal antibody (mAb) for preventing the recurrence of colorectal cancer. We evaluated its expression in myeloma cell lines and bone marrow cells from patients with multiple myeloma or primary macroglobulinemia by indirect immunofluorescence assays. Antigen 17-1A was shown positive in 3 out of 4 cell lines tested and in 8 out of 9 bone marrow cell specimens including 6 multiple myelomas, one IgG-producing chronic lymphoproliferation and 2 primary macroglobulinemias. These data suggest that administration of mAb 17-1A may be a new and feasible immunotherapy to those indolent, but incurable diseases.

Introduction

The mouse monoclonal antibody (mAb) 17-1A against a colorectal cancer-associated antigen has recently been shown effective for preventing the recurrence of colorectal cancer[1]. After a median follow-up of 5 years, mAb 17-1A therapy reduced the overall death rate by 30% and the recurrence rate by 27% in patients with colorectal cancer of stage Dukes' C after curative surgery. Although this result is similar to the benefit obtained in radiochemotherapy trials, the lesser toxicity of mAb is notable in a viewpoint of the patient's quality of life.

Most of multiple myeloma are considered to be an indolent disease predominantly occurring in the elderly. Even though its disease progression seems to be rather slow and less aggressive than other hematologic malignancies, complete cure could hardly be achieved by standard chemotherapy. Furthermore, the availability of high-dose chemotherapy and bone marrow transplantation is limited to the younger patients.
There have been few reports on the clinical study of immunotherapy of multiple myeloma. A clinical trial with anti-interleukin-6 (IL-6) mAb to neutralize the excess of IL-6, which is a putative deleterious factor in multiple myeloma, has recently been performed in 10 advanced cases[2]. Although the reduced growth rate of myeloma cells in the bone marrow was seen in 3 patients, only one patient achieved a significant regression of tumor mass. Adverse events of worsening of thrombocytopenia and neutropenia were observed in most of the patients tested. These suggest that the immunotherapy with mAb

Table 1. Expression of antigen 17-1A in cell lines and bone marrow cells from multiple myeloma patiens

Cell lines									
Cell line						Cell surface marker (%)[a]			
						CD19	CD38[b]	PCA-1[c]	17-1A
RPMI8226[d]						0.8	97.6	93.1	21.0
KR12[d]						86.5	38.2	96.5	45.6
TAPC[d]						43.2	3.8	97.3	2.5
RPMI1788[e]						58.3	17.4	68.2	12.5
Bone marrow cells									
Case no.	Age	Sex	Type	Stage	%myeloma cells[f]	Cell surface marker (%)[a,g]			
						CD19	CD38	PCA -1	17-1A
1-1[h]	51	F	IgGκ	III A	65.6	0.4	82.5	65.7	69.1
1-2[i]					0.4	0.9	48.8	17.7	9.0
2	74	F	IgGκ	II A	25.4	3.2	51.4	25.1	24.7
3	72	F	IgGκ	II A	29.9	0.6	95.2	79.5	41.8
4	69	F	IgGκ	III A	59.5	1.9	80.2	9.3	10.5
5	68	M	BJκ	II A	33.7	3.2	81.1	36.2	21.8
6	55	M	BJκ	II A	12.3	2.0	56.6	24.1	16.7
7[j]	78	F	IgGλ	I A	44.5[k]	24.7	15.6	58.6	38.7
8[l]	67	M	IgMκ		47.0[k]	44.6	17.1	52.9	51.1
9[l]	61	F	IgMλ		51.7[k]	47.2	11.3	73.5	56.6

[a]For indirect immunofluorescense, mAb CD19, CD38, PCA-1 or 17-1A and FITC-conjugated rabbit anti-mouse Ig were used as the firsy and second antibody, [b]CD38 is expressed in myeloma cells as well as in activated T cells, monocytes, preB cells and hematopoietic precursor cells. [c]PCA-1 is expressed in myeloma cells as well as in mast cells, granulocytes and macropahges. [d]Mye loma cell lines. [e]IgM-producing B cell line. [f]Number of myeloma cells / number of all nucleated cells in specimens of aspirated bone marrow cells stained with May-Giemsa. [g]After red blood cell removal under the low osmotic pressure conditions, mononuclear cells were selected with the gate and analysed. [h]Before chemotherapy. [i]After chemotherapy. The data of this specimen is shown as a negative control. [j]A case of IgG-producing chronic lymphoproliferation. [k]% lymphocytic or lymphoplasmacytic cells. [l]A case of primary macroglobulinemia.

alone in advanced multiple myeloma might be of no reality.

Bergsagel et al[3]. previously demonstrated the expression of antigen 17-1A by using another mAb GA733 in a human myeloma cell line, RPMI8226. However, there have been no reports expanding this point. We therefore examined the reactivity of mAb 17-1A with cultured myeloma cells and bone marrow cells of multiple myeloma patients by indirect immunofluorescence assays.

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 μg/ml ampicillin, 100 μg/ml kanamycin and 10% (v/v) FCS. RPMI8226, KR12 and TAPC are myeloma cell lines and RPMI1788 is a IgM-producing B cell line as previously described [4]. Human bone marrow specimens were obtained from the patients treated in Sapporo Medical University Hospital and were subjected to the analyr two-color flow cytometry, after incubation of cells with PE-conjugated anti-CD38 mAb, cells were washed twice with PBS and the indirect immunofluorescence assay for antigen 17-1A was done as described

above.

Results and discussion

As shown in Table 1, antigen 17-1A was expressed in 2 out of 3 myeloma cell lines and one IgM-producing B-cell line together with conventional myeloma markers, CD38[6] and PCA-1[7]. These confirmed an original finding on RPMI8226 cells by Bergsagel et al[3]., and suggested the expression of antigen 17-1A on tumor cells of primary macroglobulinemia. In our preliminary clinical data, 6 out of 7 myeloma specimens (85.7%) (case no. 1-3, 5, 6 and 7) were considered to be positive for mAb 17-1 based on the comparison of 17-1A-positive cells (%) with myeloma cells (%) and PCA-1-positive cells (%) in the bone marrow. Furthermore, as expected from the data on cell lines, antigen 17-1A was expressed in bone marrow cells from patients with primary macroglobulinemia (case no. 8 and 9 in Table 1). In this case, increased CD19 and decreased CD38 expressions were observed. The reactivity of mAb 17-1A with bone marrow myeloma cells in those specimens was also confirmed by two-color flow cytometry. Case no. 7 was considered to be chronic lymphoproliferation with t(11;14) (q13;q32) producing IgGl M-protein[8], in which tumor cells were morphologically similar to chronic lymphocytic leukemia, showing surface IgG-positive and overexpression of cyclin D1. Compared to typical myeloma cases (no. 1-6), the expression levels of CD19 and CD38 were increased and decreased, respectively in this case, a finding that indicates a maturation level of tumor cells between myeloma and macroglobulinemia.

Antigen 17-1A has not thus far been detected in other hematopoietic tumor cell lines, normal or activated peripheral blood lymphocytes, normal granulocytes and erythrocytes[3], although the more detailed informations on its expression in hematopoietic cells will be required. Fortunately, no serious adverse effects and no hematological toxicities of mAb 17-1 A were reported in the German study[1]. Furthermore, several lines of evidences suggested the efficacy of cytokines including now clinically available ones that enhances the antibody-dependent cell-mediated cytotoxicity with mAb 17-1A[9]. Thus, mAb 17-1A may offer a new and feasible immunotherapy to at least some part of multiple myeloma and primary macroglobulinemia patients.

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