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Abstract

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DETECTION OF DR ANTIGEN ON LEUKEMIC CELLS FROM A PATIENT SUFFERING FROM ADULT T-CELL LEUKEMIA AND PROGRESSIVE SYSTEMIC SCLEROSIS

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Abstract. This report concerns an unusual case of adult T cell leukemia (ATL) complicated with progressive systemic sclerosis (PSS). The surface markers of peripheral blood mononuclear cells (PBM) and lymphnode cells, both of which mainly consisted of leukemic cells, were examined. The effect of these cells on the pokeweed mitogen (PWM)-induced IgG synthesis by normal PBM also was studied. The leukemic cells formed rosettes with sheep red blood cells (SRBC ; E) and expressed T cell antigen, Leu-1, and DR antigen. The detection of cell surface antigens was carried out by employing monoclonal antibodies against these antigens. We diagnosed this case as DR positive ATL. In terms of the immunoregulatory function of these leukemic cells, the co-culture experiments showed that these cells had some suppressive effect on the PWM-induced IgG production by allogeneic normal PBM.

Key words : ATL, DR antigen (s), auto-immune disease.

Lymphocytes have been divided into several subsets by some sophisticated methods (1, 2) to study *in vitro* immune responses accomplished by subtle cellular interactions among these subsets. Each subset seems to have its own specific surface antigens (3) and these antigens are used to detect the subsets by using allo- and heteroantisera raised against them (4, 5). Among these surface antigens, Ia antigens encoded by genes located in the I-region of the H-2 complex are found in mice (3), and thought to play very important roles in the immune network (3). The human counterpart of Ia antigens has been detected on B cells, macrophages and activated T cells (6). These antigens have been termed Ia-like antigens or HLA-DR antigens (7) and are supposed to be important in the immune response of humans, as well.

Recently, Kohler and Milstein (8) have developed a technique of producing

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myeloma lymphocyte hybrid cell lines. This discovery offers us a way of obtaining monoclonal antibodies reactive with a variety of human lymphocyte cell surface antigens which makes it easier to detect various lymphocyte subsets (9).

In some studies (10), it has been recognized that a defect in a certain subset of lymphocytes positively relates to the triggering of immune dysfunction resulting in the development of auto-immune diseases.

On the other hand, several cases of ATL in Japan have been reported in which the leukemic cells had Ia-like antigens on their surface (11) and suppressive activity on the lymphocyte proliferating response (12). Yamada (13) reported, however, that most leukemic cells bore T cell specific antigens, but not so many bore DR antigen on their surface in a study employing specific monoclonal antibodies. He also showed with a plaque forming cell assay that the leukemic cells had potent suppressor cell activity on PWM-induced B cell differentiation. To examine whether or not a similar phenomenon could be recognized in our ATL case, we studied the cell surface antigens by employing monoclonal antibodies, and the immunological function of leukemic cells of ATL by co-culturing with normal PBM. The results confirmed the existence of DR antigen on almost all of the leukemic cells and revealed some suppressive activity of these cells on the PWM-induced IgG production. As our case of ATL was complicated with PSS, the relationship between DR positive ATL and combined auto-immune diseases was discussed.

MATERIALS AND METHODS

Case report. A 52-year-old female was admitted to the clinic of internal medicine 3 because of high fever and dyspnea of a few weeks duration. The patient, who was born in Okayama prefecture, had been healthy except for having Raynaud's phenomenon, which was diagnosed by a vascular surgeon at Okayama University Hospital about 11 years ago, and having received a thorax sympathectomy. A physical examination revealed Raynaud's phenomenon, insidious swelling of the acral portions of the extremities and tight skin of the fingers. Tightness of the skin was symmetric and was confined to the fingers (sclerodactylia). The skin of the chest wall and extremities had become taut, shiny, and hyperpigmentation was noticed especially on the forearms and lower legs. From these physical findings, the diagnosis of PSS was made. Marked enlargement of cervical, supraclavicular, and axillary lymph nodes were noted, and the spleen was palpable 3 cm below the left costal margin, but hepatomegaly was not present. These abnormalities had not been pointed out before. A mediastinal mass was observed in a chest X-ray. She died two weeks later from heart failure which suddenly developed.

Her peripheral blood leucocyte count ranged from 19,700/cmm to 22,100/cmm with 8-35 % abnormal lymphocytes. A bone marrow aspiration revealed 48 % abnormal lymphocytes. These abnormal cells frequently had a lobulated or indented nucleus. No anti-ATLA antibody was detected (checked by Prof. Dr. I. Miyoshi of Kochi Medical College). The presence of chromosomal abnormalities of malignant cells was not examined.

An axillary lymphnode specimen was obtained by biopsy. Histologically, malignant cells proliferated the specimen diffusely and no follicular formation was noted. Cytologically, the cells varied in size from 10 μ to over 20 μ in diameter. The deformed nuclei showed convolut-

ion and lobulation. Some large malignant cells had a round or oval nucleus with fine chromatin, thin nuclear membrane, distinct nucleoli and wide cytoplasm. A thymus specimen obtained by necropsy showed massive infiltration by malignant cells. Ultrastructurally, the malignant cells had markedly deformed nuclei and clustered dense bodies in the cytoplasm. These findings satisfied the criteria (14) of the diagnosis of pleomorphic T cell leukemia.

Cell preparation and detection of cell surface antigens. PBM were obtained from defibrinated venous blood by the Ficoll-Conray gradient method as previously described (15). Mononuclear cells of the lymphnode were prepared by mincing an excised lymphnode in medium RPMI-1640 and centrifuging the preparation on a Ficoll-Conray cushion after removing tissue debris by passing the cell suspension through sterilized gauze.

The cell surface of these leukemic cells was characterized by E rosetting, EA rosetting, EAC rosetting and a membrane immunofluorescent technique employing fluorescein isothiocyanate (FITC)-labelled polyclonal rabbit anti-human immunoglobulin sera (Behringwerke) and FITC-labelled monoclonal mouse anti-human T cell antigen, Leu-1 antibody (Becton-Dickinson) and FITC-labelled monoclonal mouse anti-DR antigen (Becton-Dickinson) according to a method described elsewhere (16).

Cell fractionation. The methods of cell separation have been described elsewhere (16). Briefly, E rosettes were prepared by mixing 10 ml of 3 % SRBC, 10 ml of cell suspension at a density of 3×10^5 /ml and 2 ml of fetal calf serum (FCS) absorbed to SRBC. The mixture was incubated at 4 °C for 60 min after centrifugation at 200 g for 5 min. The rosetted fraction (E-RFC) and non-rosetted fraction (non-E-RFC) were obtained by the Ficoll-Conray gradient method.

Co-culture effect of the leukemic cells on the PWM-induced IgG synthesis. Each cell fraction was suspended at a density of 1×10^6 /ml in medium RPMI 1640 supplemented with 10 % FCS, penicillin G (100 u/ml) and streptomycin (100 µg/ml). To test the helper activity of leukemic cells, 0.2 ml of the B cell rich fraction from normal PBM and 0.8 ml of the T cell rich fraction from normal or patient PBM were placed into 17 × 100 mm culture tubes (Falcon, *3033) and co-cultured for 9 days in the presence of PWM 10 µl/ml. To determine the suppressive effect of leukemic cells on the PWM induced IgG production, 1×10^6 cells of normal PBM and 2×10^5 or 5×10^5 leukemic T cells were co-cultured for 9 days with PWM 10 µl/ml and the amount of IgG synthesized in the culture supernatant was analyzed with the double antibody radio immunoassay for IgG as previously described (15).

RESULTS

Characterization of leukemic cells. Patient PBM contained 72.8 % E-RFC, 9.0 % surface immunoglobulin (s-Ig) positive cells, 11.5 % EA-RFC and 5.4 % EAC-RFC, as shown in Table 1. Leukemic cells as defined cytologically formed E rosettes, but not EA or EAC rosettes. This result suggests that this leukemia is of T cell origin.

As Table 1 shows, 68.1 % of the axillary lymphnode cells were malignant cells. 69.4 % were E-RFC, 9.0 % were S-Ig (+) cells, 23.8 % were Leu-1 positive cells (40.0 % of the malignant cells) and 50.3 % were DR positive cells (87.5 % of malignant cells). These results confirmed that this leukemia was of T cell origin and expressed T cell antigen Leu-1 and DR antigen on their surface.

Effect of leukemic cells on the PWM induced IgG production. As described above,

TABLE 1. CHARACTERIZATION OF LEUKEMIC CELLS

Characteristic	PBM	Lymphnode cells ^a
E-rosetting	72.8 (%)	69.4 (%)
EA-rosetting	11.5	n.d ^b
EAC-rosetting	5.4	n.d
S-Ig (+)	9.0	9.0
Leu-1 (+)	n.d	23.8 ^c
DR (+)	n.d	50.3 ^a

a: Lymphnode cells consisted of 68.1% malignant cells. *b*: Not done. *c*: 40.0% of the malignant cells were Leu-1 (+). *d*: 87.5% of the malignant cells were DR (+).

TABLE 2. HELPER EFFECT OF LEUKEMIC T CELLS

Cell source of co-culture ^a		Amount of IgG ^b (ng/ml)
B cells	T cells	
Normal (1)	Normal (2)	2,300
Normal (2)	Normal (1)	1,900
Normal (1)	Leukemic	0
Normal (2)	Leukemic	0
Leukemic	Leukemic	0

a: 2×10^5 B cell rich fraction and 8×10^5 T cell rich fraction were co-cultured in 1 ml culture medium containing $10 \mu\text{l}$ PWM. *b*: IgG produced in the culture supernatant was measured by a double antibody RIA.

TABLE 3. SUPPRESSIVE EFFECT OF LEUKEMIC T CELLS^a

(-)	Cells added ^b	
	Leukemic T (2×10^5)	Leukemic T (5×10^5)
2,500 ^c	1,440	615
4,600	4,800	2,250
1,900	2,100	540
2,900	4,300	2,400

a: Effect of leukemic T cells on the PWM-induced IgG production by normal PBM (1×10^6) was examined by co-culturing with two different numbers of leukemic T cells. *b*: 1 ml of normal PBM ($1 \times 10^6/\text{ml}$) and 0.2 ml or 0.5 ml of leukemic T cells ($1 \times 10^6/\text{ml}$) were co-cultured with $10 \mu\text{l}/\text{ml}$ PWM. *c*: Each figure expresses the amount of IgG secreted as ng/ml.

leukemic cells expressed T cell and DR antigens. In order to determine whether these leukemic cells had any helper or suppressive function in the immune response, co-culture experiments were carried out. The normal B cell-rich fraction and normal or leukemic T cell-rich fraction were co-cultured at a 1 : 4 ratio in 1 ml of medium in allogeneic fashion, and the mixture was analyzed for secreted

IgG after 9 days of incubation. As shown in Table 2, normal T cells helped allogeneic normal B cells to synthesize IgG in response to PWM, but leukemic T cells had no helper effect on the normal B cell response.

Co-culture experiments were also carried out to determine whether these leukemic cells had any suppressive effect on the PWM-induced IgG production. As shown in Table 3, 2×10^5 leukemic T cells did not suppress the PWM-induced IgG secretion by normal PBM, but, 5×10^5 of them markedly suppressed it.

These data indicate that leukemic cells had no helper function but may have some suppressive effect on the PWM induced IgG synthesis by normal PBM.

DISCUSSION

The present study confirms the observations by Hattori *et al.* (11) that Japanese adult T cell leukemic cells possess human B lymphocyte antigens (HBLA) which are thought to be the human counterpart of Ia antigens in mice, and are called Ia-like antigens or DR antigens (7). They detected these Ia-like antigens on leukemic cells by employing commercially available rabbit anti-HBLA serum. Although the reactivity of this serum was specific against HBLA, this serum seemed to contain many non-specific antibodies, as well. Even if regarded as having specific antibodies, the reagent consisted of polyclonal antibodies which react with many epitopes of the Ia-like antigens. To eliminate the cross reactivity of these antibodies, the best way is supposed to be the detection of a single epitope of B cell antigens by the use of a monospecific antibody. Fortunately, Kohler and Milstein (8) made it possible to obtain monoclonal antibodies against a certain antigen by establishing the myeloma lymphocyte hybrid cell lines. Yamada (13) employed monoclonal anti-DR antibody to confirm the presence of DR antigen(s) on leukemic cells of ATL. His investigation showed some anti-DR antibody reactive leukemic cells in 5 out of 16 leukemia patients, but the percentage of DR antigen positive cells was very low, 30.5 % at most. Our investigation revealed slightly different results. As shown in Table 1, almost all leukemic cells possessed DR antigen. In other countries, several investigations (17) were done to detect DR antigen on cells of T cell malignancy using monoclonal antibodies against DR antigen. They reported, however, that they failed to prove the existence of DR antigen on cells of T cell malignancy. From these data and ours, it may be said that Japanese ATL is a new entity of T cell malignancy since some investigators (14, 18) reported that Japanese ATL had an unusual clinical course and the leukemic cells had particular morphological characteristics.

Generally, it has been accepted that lymphoid cell malignancies originate from certain lymphocyte subsets which may have their own specific functions, and there have been reports dealing with aspects of this idea, e.g., CLL with monoclonal gammopathy, Sezary syndrome with helper T cell function (19) and ATL with suppressive function (20) in *in vitro* immunoglobulin synthesis. Moreover, DR

antigens play a very important role in the immune circuit (21). It is very reasonable, therefore, to assume that leukemic cells with DR antigen may exert some regulatory function in the immune response. As shown in Table 2, we detected no helper function, but, as shown in Table 3, some suppressive effect on the PWM induced IgG production was noted in the presence of 5×10^5 leukemic cells in a co-culture system. It is very difficult to reach the conclusion that these leukemic cells with DR antigen have a suppressive effect, because an overcrowding effect in co-culture has not been denied and 2×10^5 leukemic cells did not suppress IgG production. In addition, Yamada (13) and Nakahara *et al.* (22) reported that leukemic cells in ATL did not express the surface phenotype of suppressor/cytotoxic T cells.

As mentioned above, DR antigen are necessary for immune regulation. Therefore, it can be assumed that DR positive leukemic cells with immunological function cause an immunologic aberration resulting in development of auto-immune diseases, such as systemic lupus erythematosus and PSS. Since the appearance of PSS symptoms preceded the development of ATL by several years in our case and we have not found any reports which deal with DR positive ATL complicated with auto-immune diseases so far, the relationship between DR positive ATL and combined auto-immune diseases remains unclear.

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