Expression of CD3 Antigen on Individual Adult T Cell Leukemia Cells Detected by Immunocytochemistry

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Adult T cell leukemia (ATL) is a T cell malignancy caused by human T cell leukemia virus type I (HTLV-I) infection [1], and is characterized by the proliferation of abnormal lymphocytes with deeply indented or lobulated nuclei, and with a mature CD4-positive phenotype [2].

Characterization of ATL cells using monoclonal antibodies (mAbs) has revealed several biological features. A series of mAbs to the cell surface membrane revealed that the mode of activation of ATL cells differs between clinical stages [3]. In particular, the decrease in CD3 antigen expression and the increased expression of CD25 antigen on ATL cells analyzed by immunofluorescence have been reported to be specific to ATL [4]. However, in previous studies, the expression of CD3 antigen on ATL cells has been analyzed by flow cytometry using peripheral blood lymphocytes from patients with ATL. This kind of immunofluorescence study could not detect the changes in surface antigens specifically limited to ATL cells themselves, because ATL and other cells were analyzed together. In the present study, we examined the expression of CD3 antigen on morphologically identified fresh ATL cells by immunohistochemistry. Seventeen patients were diagnosed as positive for ATL and were clinically subtyped based on the criteria defined by the Lymphoma Study Group [5]: 8 patients had acute-type ATL, 4 had lymphoma type, 2 had chronic type, and 3 had smoldering type. Clinical parameters such as serum lactate dehydrogenase (LDH) and Ca levels, the numbers of WBC and peripheral abnormal lymphocytes were also studied. Peripheral blood of patients was smeared on a glass slide, air-dried, and then smears were stained by the avidin-biotin-peroxidase complex (ABC) method [6] using an ABC kit (Vector Laboratories, Burlingame, Calif., USA) with Leu4 mAb (IgG1 anti-CD3 mAb, Becton Dickinson Immunocytometry Systems, Mountain View, Calif., USA). Stained smears were further stained with Giemsa solution according to the double-staining method of Mori et al. [7] to microscopically identify ATL cells by their morphological characteristics (fig. 1). In practice, we defined the CD3-positive ATL

cells as those whose whole cell surface was diffusely stained by anti-CD3 mAb. Expression of CD3 antigen is shown as the percentage of CD3-stained ATL cells in all ATL cells (%CD3) which were morphologically identified. Eight healthy adults were used as the control group to examine the sensitivity of CD3 staining by the ABC method. The CD3-positive cells, as determined by the ABC method, comprised $72.8 \pm 4.0\%$ of peripheral mononuclear cells (PMNCs), which is compatible with the CD3-positive cells in PMNCs in healthy adults analyzed by flow cytometry using the same mAb [8]. Thus, the ABC method showed intensity similar to that determined by flow cytometric analysis.

The %CD3 of ATL cells ranged from 46.0 to 98.0% and showed significant inverse relationships with serum LDH values ($r = -0.80$, $p < 0.0005$; fig. 2). There were no morphological differences between ATL cells that stained with anti-CD3 mAb and those that did not. It was difficult to find a correlation between clinical stages and %CD3, partly because there were too few patients. Although we could not analyze the same PMNCs from the present patient group, no such significant inverse relationship between the fluorescence intensity of CD3 analyzed by

Fig. 1. ATL cells stained with anti-CD3 mAb by the ABC method. Fig. 2. Correlation between expression of CD3 antigen on ATL cells and serum LDH levels. The correlation coefficient was -0.80 ($p < 0.0005$).

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Fig. 3. Comparison of WBC counts and percentages of abnormal cells between patients analyzed by the ABC method and those by flow cytometry (FCM). a WBC count, b Percentage of abnormal cells in the peripheral blood.

flow cytometry and serum LDH levels was observed in another group of 39 patients with ATL on a separate occasion ($r = -0.39$, $p < 0.05$). To further examine why the inverse relationship has not been found following flow cytometric analysis, we compared the data on WBC numbers and percentages of abnormal cells between patients

studied by the ABC method and those by flow cytometry (fig. 3). No significant difference was observed between the two groups either in WBC number or percentage of abnormal cells. In addition, no significant difference was observed when the patients with the same ATL subtype were compared between the two groups. These results

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supported the idea that the inverse relationship between %CD3 and LDH levels reflected the expression of CD3 antigen specifically limited to ATL cells themselves.

The down-regulation of the CD3/T cell receptor (TCR) complex is known to be associated with the activation of T cells. Indeed, the modulation of CD3 antigen density and enhancement of CD25 antigen expression were caused by the incubation of fresh ATL cells with anti-CD3 mAb and were closely related in cells from ATL patients, regardless of their clinical stages [9]. In addition, among cell lines established from patients with ATL, only those originating from primary leukemic clones in vivo expressed a low density of the CD3-TCR complex [10]. Further investigation of phenotypic and functional changes following HTLV-I infection of a T cell clone over a long period revealed that from a period of about 200 days after HTLV-I infection, the cells proliferated strongly in the absence of interleukin-2 and had lost all functional activity. These cells did not express the CD3/TCR receptor complex on their surface [11]. These findings support the idea that infection by HTLV-I was not necessarily associated with decreased expression of the CD3/TCR complex. Therefore, the down-regulation of CD3 antigen expressed as %CD3 of ATL cells in our patients may be more closely related to the state of leukemic cells induced by HTLV-I infection, and the down-regulation of the CD3-TCR complex on ATL cells may play a key role in the development of HTLV-I-induced leukemogenesis in ATL. The mechanisms of down-regulation of the CD3-TCR complex are still unclear. It was reported that the T-cell-specific CD3e enhancer was inactive in an HTLV-I-infected T cell clone which could be cultured in the absence of interleukin-2 and lacked CD3 proteins [12].

Prognosis of adult patients with ATL has been reported to be generally poor. Analysis of prognostic factors in patients with ATL revealed that among serum biochemical parameters, high LDH levels and hypercalcemia were associated with shortened survival [13]. The serum LDH level is known to be increased in many malignant neoplastic diseases of the hematopoietic system, mainly because of an increased turnover of malignant cells [14]. Although the %CD3 of ATL cells in our patients showed no direct association with serum Ca levels, the mean %CD3 positivity was higher in patients with than in those without hypercalcemia ($p < 0.05$). Patients without hypercalcemia showed significantly longer survival times than those without (2.3 vs 7.6 months, $p < 0.005$).

In conclusion, the expression of CD3 antigen on individual ATL cells may be one of the factors reflecting the pathophysiological state of ATL. The biological function of CD3 expression on ATL cells remains to be elucidated.

References

Yoshida M, Miyoshi I, Hinuma Y: Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci USA* 1982;79:2031-2035. Hattori T, Uchiyama T, Toibana T, Takatsuki K, Uchino H: Surface phenotype of Japanese adult T-cell leukemia cells characterized by monoclonal antibodies. *Blood* 1981;58:645-647.

Shirono K, Hattori T, Hata H, Nishimura H, Takatsuki K: Profiles of expression of activated cell antigens on peripheral blood and lymph node cells from different clinical stages of adult T-cell leukemia. *Blood* 1989;73:1664-1671. Tsuda H, Takatsuki K: Specific decrease in T3 antigen density in adult T-cell leukemia cells. *Br J Cancer* 1984;50:843-845. Shimoyama M and members of The Lymphoma Study Group (1984-1987): Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia and lymphoma. *Br J Haematol* 1991;79:428-437.

Hsu SM, Raine L, Fanger H: Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 1981;29:577-580.

Mori M, Zaïke Y, Fujiwara M, Mori S: Identification of lymphocyte surface marker on blood smear. *Jpn J Clin Hematol* 1985;26:717-720.

Goto M, Miyamoto T, Nishioka K, Uchida S: T cytotoxic and helper cells are markedly increased, and T suppressor and inducer cells are markedly decreased, in rheumatoid synovial fluids. *Arthritis Rheum* 1987;30:737-743.

Matsuoka M, Hattori T, Chosa T, Tsuda H, Kuwata S, Yoshida M, Uchiyama T, Takatsuki K: T3 surface molecules on adult T cell leukemia cells are modulated in vivo. *Blood* 1986; 67:1070-1076.

10 Shirono K, Hattori T, Matsuoka M, Matsushita S, Asou N, Takatsuki K: Adult T cell leukemia cell lines that originated from primary leukemic clones also had a defect of expression of CD3-T cell receptor complex. *Leukemia* 1988; 2:728-733.

Yssel H, de Waal Malefyt R, DucDodon M, Blanchard D, Gazzolo L, de Vries JE, Spits H: Human T cell leukemia/lymphoma virus type I infection of a CD4⁺ proliferative/cytotoxic T cell clone progresses in at least two distinct phases based on changes in function and phenotype of the infected cells. *J Immunol* 1989; 142:2279-2289.

de Waal Malefyt R, Yssel H, Spits H, de Vries JE, Sancho J, Terhorst C, Alarcon B: Human T cell leukemia virus type I prevents cell surface expression of the T cell receptor through down-regulation of the CD3- γ , - δ , - ϵ , and - ζ genes. *J Immunol* 1990;145:2297-2303.

Lymphoma Study Group (1984-1987): Major prognostic factors of patients with adult T-cell leukemia and lymphoma: A cooperative study. *Leuk Res* 1991;15:81-91.

Drexler HG, Gaedicke G, Minowada J: Enzyme markers in acute leukemias: Advances during the last decade. *J Natl Cancer Inst* 1984; 72:1283-1298.