Short Communication

Specific decrease in T3 antigen density in adult T-cell leukaemia cells: I. Flow microfluorometric analysis

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Adult T-cell leukaemia (ATL) cells have characteristics of neoplasms originating in fairly well differentiated T-cells (Takatsuki *et al.*, 1982). Phenotypically, they express OKT 3⁺ 4⁺ 10⁺ 11⁺ 17⁺ 5⁻ 6⁻ 8⁻ uniformly implying a common origin of the tumour cells (Hattori *et al.*, 1981; Tsuda & Takatsuki, 1983 *a, b, c*). However, we have occasionally encountered patients with a reduced number of T3 cells in comparison with that of T4⁺ or T11⁺ cell (Tsuda & Takatsuki, 1983*a, b, c*). In this study, using flow microfluorometry, we reevaluated not only the percentage of antigen (Ag) positive cells but also the Ag density on these cells. The T3 density was found to be dramatically and specifically reduced in ATL cells. Recently, many reports revealed that the T3 molecule is involved in T-cell recognition or triggering by antigen (Beverley, 1983). Significance of our observation, along with some additional experiments, will be discussed with respect to T cell activation and transformation.

The diagnoses of patients (ATL 12, T cell-chronic lymphocytic leukaemia (T-CLL) 1) were based on accepted clinical, haematological and laboratory findings. Anti-ATLA antibody (antibody to ATL-virus associated Ags) in sera examined by indirect immunofluorescence (Hinuma *et al.*, 1981) and HTLV (human T cell leukaemia virus) proviral DNA in leukaemia cells (Poesz *et al.*, 1980; Yoshida *et al.*, 1982) were positive in all ATL and negative in T-CLL. Twenty-one healthy laboratory personnel ranging in age from 23 to 40 years were studied to establish reference ranges. Cord blood from 5 healthy newborns was also utilized.

Peripheral blood mononuclear cells from both patients and controls were separated from heparinized blood by Ficoll-Conray density gradient centrifugation. T cells were obtained by a single centrifugation of neuraminidase-treated sheep erythrocyte (En) rosettes through a Ficoll-Conray

density gradient (En rosette method) followed by lysis of attached En with 0.01 M Tris-0.83% NH₄Cl buffer. Cell viability was assessed by trypan blue exclusion and was always >90%.

Indirect or direct immunofluorescent analysis of cell preparations was performed as follows. In indirect method, 10⁶ cells (100 µl) were incubated with 10 µl of OKT9 (Ortho) (Reinherz & Schlossman, 1980) or 100 µl of 1/2000 diluted anti Tac ascitic MoAb (provided by Dr. Uchiyama) (Uchiyama *et al.*, 1981) for 30 min on ice. The cells were then washed twice with RPMI-1640. This was followed by incubation with 100 µl of 1/80 diluted fluorescein isothiocyanate (FITC)-goat anti-mouse IgG antibody (G/M FITC; Cappel) for 30 min on ice. Direct staining was assessed by incubating 10⁶ cells (100 µl) with 10 µl of FITC-labelled MoAbs (OKT3, OKT4, OKT8, OKT11) (Ortho) (Reinherz & Schlossman 1980; Verbi *et al.*, 1982) for 30 min on ice. In both methods, after washing twice with PBS, cells were analysed for fluorescence on a laser flow cytometry system, Spectrum III (Ortho) by exposure to a laser light of 488 nm at an intensity of 20 mW.

Paired student's t-test was used for statistical analysis of results.

Analysis of surface phenotype revealed that ATL cells from all patients except Patient 8, which was a lymphoma type, reacted positively with OKT3, T4, T11 MoAbs; no reactivity was noted with OKT8 (Table I). However the degree of OKT9 and Tac Ag expression was varied. In contrast with ATL, T-CLL cells reacted with OKT3, T8, T11 but not with OKT4 (Table I). These cells were OKT9 positive and Tac negative in three repeated analyses. Simultaneously, fluorescence intensity (FI) of cells stained for each MoAb was analysed. The results for OKT3, T4 and T11 are shown in Figure 1. No significant difference was found in FI for T4 (79.2 ± 26.6 and 71.5 ± 12.2 , $0.4 < P < 0.5$) or T11 (69.6 ± 28.8 and 57.5 ± 26.2 , $0.2 < P < 0.4$) between ATL and normal T cells, though ATL cells had varied density of these Ags. On the contrary, FI for T3 was markedly decreased in ATL (40.2 ± 16.1) in comparison with normal T cells (111.4 ± 13.0)

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Table 1 Surface Phenotype of T-cell leukaemia Cells.

Patient	No.	Age (Yr.)	Sex	WBC ($\times 10^9/L$)	Reactivity with Monoclonal Antibodies (%)					
					T3	T4	T8	T11	T9	Tac
ATL	1	35	M	14.4	94.2	93.7	3.1	96.3	9.7	48.0
	2	68	F	10.4	72.9	71.9	3.6	84.6	4.2	3.4
	3	51	F	16.0	89.8	85.0	7.4	94.6	7.0	32.1
	4	56	F	46.0	81.6	91.1	3.4	96.8	2.2	6.1
	5	71	F	14.9	62.6	90.1	3.1	93.9	43.8	32.7
	6	74	M	53.8	75.5	98.3	5.4	99.4	29.5	44.9
	7	62	F	16.6	90.9	91.6	2.6	93.3	14.5	43.7
	8	56	F	12.1	76.3	67.7	46.1	93.8	—	—
	9	70	M	120.0	76.9	96.6	7.8	98.7	25.6	40.4
	10	52	M	96.2	43.2	93.1	2.3	93.0	0.0	14.3
	11	76	F	139.0	78.4	76.0	7.2	81.5	—	—
	12	44	M	91.2	91.3	86.4	3.1	91.5	—	—
T-CLL		38	F	22.0	98.4	7.7	90.5	98.7	24.0	0.2

Figure 1 Density of OKT antigens on ATL cells. T3, T4 and T11 antigens on ATL (●) and T-CLL(X) cells were stained by the direct immunofluorescence method and assayed for fluorescence intensity(FI) by flow microfluorometry. FI is expressed as arbitrary channel unit (mean channel, linear scale). Dotted area indicates FI in normal T cells (Mean $\pm$ sd).

($P < 0.001$). T-CLL showed a slightly higher FI than normal T cells in several tests (Figure 1). Fluorographic pattern for T3 (Figure 2) consistently displayed a narrow peak of ATL cells corresponding to the lower part of broad peak of normal T cells.

There are several possible explanations for these findings: (a) Specific reduction of T3 but not of T4

Figure 2 Fluorescence histogram of ATL and normal T cells stained by FITC-OKT3 antibody. In order to display the difference in patterns of histogram, ATL 2 (Table 1) and normal T cells were stained for T3 antigen, mixed (1:1), and analysed by flow microfluorometry. The narrow peak of ATL cells is seen on the left shoulder of the broad peak of normal T cells.

or T11 implies that aberrant T3 expression nonspecifically caused by malignant transformation is unlikely. (b) An activated state of ATL cells does not explain this observation because conA-activated normal T cells express T3 much more than fresh T cells (data not shown). (c) Patients' sera may contain a factor(s) that blocks the binding of T3 MoAb to Ag or inhibits the expression or T3.

However, the combined incubation (for 2 h, at 4°C) of sera (50%) and cells, which were cultured in RPMI-1640 with 10% FBS for 3 days at 37°C prior to the study, from patients and normals yielded no significant difference (data not shown). Furthermore, when we cultured the cells in media with sera (10% or 20%) from ATL patients and healthy adults for 24 h at 37°C, there were not significant differences in T3 density between cells cultured with different sera, though a slightly increased T3 density (~30%) was observed in both ATL and normal T cells (data not shown). (d) The most probable explanation hitherto is tropic infection of HTLV to a subset of T cells with T3 in low level. T3 appears on T cells in late intrathymic ontogeny, later than such Ags as T4, T8 and T11 (Reinherz & Schlossman 1980; Verbi *et al.*, 1982; Reinherz *et al.*, 1982). Moreover, the relative ease with which cord blood lymphocytes can be infected with HTLV compared to adult lymphocytes (Popovic *et al.*, 1983) suggests the possibility that the virus is tropic for a subset of relatively immature lymphocytes. Although the T3 density on cord blood lymphocytes was not unexpectedly low (98.0 ± 5.9), this possibility can not be excluded because the peak of T3 in fluorescence histogram is

rather broad in both cord and adult T cells (Figure 2). (e) Finally, structure or expression of T3 molecules may be altered by viral infection or malignant transformation. Considering the importance of T3 Ag in T cell activation and antigen recognition (Beverley, 1983; van Wauwe *et al.*, 1980; Reinherz *et al.*, 1982; Meuer *et al.*, 1983) this possibility is most interesting. T3 is rapidly induced to modulate by anti T3 via external shedding from the cells. It is also suggested that the modulation of T3 structure might activate the cells in a parallel fashion to the antigen itself; at least insofar as becoming more receptive to the second proliferative signal, interleukin-2 (IL-2) (Reinherz *et al.*, 1982). ATL cells that express Tac Ag (IL-2 receptor) (Leonard *et al.*, 1982) are known to proliferate *in vitro* in response to conditioned medium containing IL-2 (Tsuda & Takatsuki, 1983b,c). Therefore, it is intriguing to speculate that reduction of T3 density observed might correlate with activation or growth of ATL cells.

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