Alpha-foetoprotein in Chronic Lymphocytic Leukaemia*

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SUMMARY **Alpha-foetoprotein (AFP) antigen was detected by using the indirect fluorescent technique on lymphocytes from 12 patients with chronic lymphocytic leukaemia (CLL). The percentage of AFP antigen-positive cells varied from case to case and seemed to be highly increased on T-enriched lymphocytes from CLL patients. Preincubation of the patients' lymphocytes with anti-AFP antibody had an enhancing effect on the E-rosette binding capacity of the treated cells. This capacity was significantly increased by prolonged incubation of the treated lymphocytes with sheep red blood cells (SRBC) at 4°C. Preincubation of the patients' lymphocytes with anti-AFP was found to interfere with the T-cell Fc-surface receptor for IgG complexes, suggesting the possibility that the carcinofoetal antigen may be located on the cell membrane of this subpopulation of T-suppressor cells.**

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The presence of a membrane receptor for alpha foetoprotein (AFP) antigen on normal lymphocytes as well as its immunosuppressive effect on these cells[1-4] make especially interesting investigations on this antigen in lymphoproliferative disorders. Oncofoetal antigens were found in patents with Hodgkin's disease,[5,6] in cases of sarcoma,[7] and in circulating lymphocytes of patients with lymphomas.[8]

In view of some antigenic similarities between foetal and CLL lymphocytes,[9] the different distribution of the major membrane glycoproteins on the lymphocyte surface of normal and CLL T and B lymphocytes,[10] the relative immunological inertness and immunoincompetence of the circulating lymphocytes in CLL patients,[11,12] we found that it may be of interest to examine these cells for the possible presence of a membrane-bound oncofoetal antigen. The current preliminary study was initiated to investigate whether experimental evidence can be obtained on the presence of AFP antigen on lymphocytes from CLL patients. Based on the observations that AFP can suppress certain T-cell-dependent function,[2-4] the possible correlation of this antigen with the patients' T-lymphocyte markers was also considered.

MATERIALS AND METHODS

Patients

The study comprised 12 patients with CLL (Table 1). Their disease status, according to Ray's[13] clinical staging of CLL, was classified from 0 to III. Eight of the 12 patients were in a mild to moderate stage (0-I), and patients 4, 8, 9 and 12 (Table 1) in a more severe stage (II-III); three of them received anti-leukaemic treatment at the time of examination (Table 1). Seven healthy blood bank donors (male) served as controls.

Methods

The indirect fluorescent technique was used to obtain experimental evidence of AFP antigen on the patients' lymphocytes. To observe whether the antigen can be correlated with the patients' T-cell membrane, the modulating effect of anti-AFP antibody treatment in low concentration on T-cell-specific surface markers, such as spontaneous E-rosette formation with SRBCs and the T-lymphocyte Fc IgG receptor, characteristic for a subpopulation of T-suppressor cells,[14] was examined. For all tests, mononuclear cells were obtained from Hypaque-Ficoll sedimentation of peripheral heparinised blood.[15]

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Table 1 The effect of anti-AFP antibody treatment on membrane fluorescent staining and E-rosette formation of lymphocytes from CLL patients

Patients	Sex	Total WBC/μl	Lymphocytes %	Therapy	Fluorescent* %	E rosette % – 1 hour at 4°C		
						Untreated cells	Medium	Anti-AFP**
1	M	133,000	96	–	14	7	6	12
2	M	26,800	85.	–	15	9	15	20
3	M	32,000	91	–	20†	6	11	12
4	M	493,000	80	Leukeran	10	4	4	7
5	M	30,200	84	–	15	6	10	24
6	M	36,000	83	–	10	9	8	8
7	M	19,400	78	–	16	10	16	20
8	M	42,300	95	Meticorten	10	10	12	21
9	F	63,000	87	–	12†	6	8	21
10	F	47,700	86	–	19	14	13	11
11	F	14,100	60	–	18†	26	28	32
12	F	42,500	96	Meticorten	15	4	3	3

*Anti-AFP dilution 1:10

†The percentage of fluorescent staining cells in T-lymphocyte preparations was 30%, 38% and 35%, respectively.

**Anti-AFP 1:200, Student's paired "t" test for statistical evaluation. Preincubation with medium had no significant effect on the E-rosette formation. Preincubation with anti-AFP significantly enhanced the percentage of E rosettes ($P < 0.01$).

Indirect immunofluorescent studies

To demonstrate specificity of the immunofluorescence, the fluorescein-conjugated antibody (7S goat anti-rabbit IgG-Hyland) was absorbed overnight at 4°C on the respective normal or leukaemic lymphocytes. The remaining lymphocytes resuspended in RPMI 1640 supplemented with 5% inactivated normal pooled human serum were stored overnight at 37°C in 5% CO_2 humidified air, washed in phosphate buffered saline (pH = 7.3), counted, incubated for one hour with specific rabbit antihuman AFP antiserum at 37°C (Behring, 0.4 mg/ml antibody), washed and reincubated with the absorbed fluorescent anti-rabbit antiserum (concentration 1:8, 1:5). The thoroughly washed cells were examined for fluorescence under an IV F_1 epifluorescence condensor with an HBO 50W superpressure mercury lamp. For pictures, a dark-field condensor with translumination was added. For control, heat-inactivated absorbed normal rabbit serum and rabbit anti-human albumin (Behring) were used instead of anti-AFP antiserum. For anti-AFP blocking experiments, AFP human standard serum (Behring) was employed to demonstrate the specificity of immunofluorescence.

E rosettes with SRBCs

Patients' and controls' lymphocytes pretreated with anti-AFP antiserum (Behring, concentrations 1:10, 1:20, 1:200) for one hour at 37°C in a humidified atmosphere of 5% CO_2, washed, counted for viability (92-94%), were tested for their capacity to form E rosettes.[16] For each patient, untreated lymphocytes and lymphocytes treated in medium only were used for control. In a number of cases (patients and controls), the effect of a prolonged incubation time (24 hours at 4°C) for the E-rosette formation of anti-AFP treated and untreated cells was studied.

T lymphocytes with IgG receptors (Tγ cells)

In seven patients and six controls, the presence of the Fc receptor for IgG complexes was tested on enriched T lymphocytes. T lymphocytes were purified from non T cells by rosetting with SRBCs which were lysed from the lymphocytes using a hypotonic shock, a short exposure to 0.24% NaCl. The enriched T cells were incubated as previously described with anti-AFP antiserum, thoroughly washed and used for the determination of T cells by rosette formation with ox erythrocytes coated with the IgG fraction of rabbit anti-ox erythrocyte antibody.[14] Anti-ox red cell Ig antibody was raised in rabbits, purified over a Sephadex G-200 column and controlled for IgG specificity by immunoelectrophoresis.

RESULTS

Indirect immunofluorescence studies

The percentage of positive stained cells (Fig. 1) varied from case to case (Table 1), and could be observed at antibody dilutions of 1:10 and 1:20. No significant lymphocyte surface staining could be observed with inactivated, absorbed

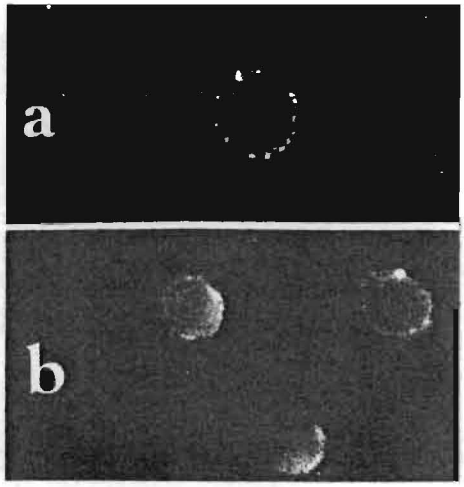

Fig. 1 a. Lymphocyte from a CLL patient showing rim membrane immunofluorescence of microaggregates.
b. Capping of T lymphocytes from the same patient.

rabbit control serum, with rabbit anti-human albumin or in blocking experiments. The percentage of stained control lymphocytes was < 4 per cent. With enriched T lymphocytes from CLL patients, a significantly increased percentage of positive stained cells could be observed (Table 1). The percentage of positive stained eariched B cells was between 5 and 7 per cent. No more than 3-4 per cent of fluorescent stained cells could be observed on enriched T or B control lymphocytes.

E rosettes with SRBCs

After preincubation with anti-AFP antibody an increase in the E-rosette-forming capacity of the patients' lymphocytes could be observed in seven out of 12 patients when compared with the same lymphocytes treated in medium only (Table 1). A prolonged incubation at 4°C with SRBCs seemed to be favourable for additional enhancement of the E-rosette-forming capacity of anti-AFP-treated CLL lymphocytes (Table 2). The E-rosette-forming capacity of control lymphocytes was not affected by anti-AFP treatment at various concentrations or by prolonged incubation with SRBCs (Table 2).

Table 2 Effect of prolonged incubation at 4°C on the E-rosette formation of anti-AFP-treated lymphocytes from controls and CLL patients.

Lymphocytes	No. of cases	E rosettes (%) after 24 hours of incubation		
		Untreated cells	Treated cells	
			Medium	Anti-AFP*
Controls	7	56.4 ± 5.2**	52.2 ± 4.1	56.2 ± 5.1
CLL	7†	11.8 ± 2.6	14.4 ± 4.0	23.5 ± 5.3

†Patients 2,3,7,8,10,11,12 from Table 1.
**Mean % ± SE.
*A dilution of 1:200 anti-AFP was found optimal for the modulating effect on E-rosette-forming lymphocytes from CLL patients. Control cells were not affected by pretreatment with anti-AFP (1:20, 1:100, 1:200). Student's paired "t" test for the evaluation of the anti-AFP effect. (Anti-AFP treated vs. untreated lymphocytes, $p < 0.05$).

T lymphocytes with Fc IgG receptors

As can be seen in Figure 2, the percentage of Fc IgG receptor bearing T lymphocytes was significantly increased in CLL patients compared with controls. After preincubation with anti-AFP antibody, the percentage of Fc IgG receptor bearing T lymphocytes from CLL patients was significantly lower than in those without treatment and was comparable to the percentage of Fc IgG receptor bearing T lymphocytes from untreated controls.

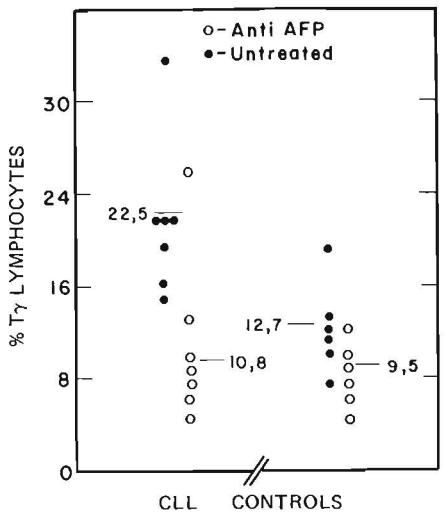

Fig. 2 Tγ lymphocytes in CLL patients and controls. Effect of anti-AFP. For CLL patients, Tγ significantly decreased after antibody treatment. ($p<0.01$). No significant difference for controls. (Student's paired "t" test).

DISCUSSION

The indirect immunofluorescence technique used in this study revealed that AFP antigen can be detected as a surface-binding antigen on some circulating lymphocytes from CLL patients. The increased percentage of stained cells in the T-cell enriched lymphocyte population of CLL patients, the low percentage of such cells in the B-cell-enriched population, the modulating effect of anti-AFP treatment on some T-lymphocyte surface markers of these patients, on spontaneous E-rosette formation and on the T-lymphocyte Fc IgG receptor, seem to suggest that AFP antigen is mainly bound to the cell surface of certain T-lymphocyte subpopulations. Although it remains unclear from this study how AFP antigen-antibody complexes can interfere with the E-rosette-binding lymphocyte receptor, it may be assumed that movement on the cell surface of AFP antigen-antibody complexes and topographic redistribution of membrane components may lead to the unmasking of the specific E-rosette receptor and to an increased binding capacity of these lymphocytes to SRBCs.[17] A prolonged incubation with SRBCs at 4°C was observed to enhance further the number of probably previously cryptic or unavailable E-rosette-binding receptor sites of anti-AFP-treated lymphocytes from CLL pa-

tients. No such enhancement could be observed with control lymphocytes.

Our observation on an increased percentage of Tγ cells in CLL patients correlates well with the observation of Gupta and Good[14] concerning increased number of Tγ cells in patients with certain types of immunodeficient disorders. Murgita *et al*[2] observed that AFP can induce suppressor T cells *in vitro*. The modulating effect of anti-AFP on the Fc IgG receptor of T lymphocytes from CLL patients observed in this study and the reduced expression of Fc IgG receptors after anti-AFP treatment suggest various explanations. These phonomena may be due to an inhibitory effect,[18] but may also be the result of a close location of the Fc IgG-complex receptor and AFP antigen binding sites on the same lymphocyte membrane surface.

AFP binding sites were suggested to be closely spaced to Con A receptor sites on murine T lymphocytes.[1] Further studies on the modulating effect of anti-AFP antibody on additional surface markers of lymphocytes from CLL patients and examinations of some immune functions of AFP-bearing lymphocytes seem to be necessary.

REFERENCES

1. Dattwyler RJ, Murgita RA, Tomasi TB Jr. Binding of alpha-fetoprotein to murine T cells. Nature 1975; 256:656-7.
2. Murgita RA, Goidl E, Kontiainen S, Wigzell H. Alpha fetoprotein induces suppressor T cells *in vitro*. Nature 1977; 267:257-9.
3. Gupta S, Good RA. α fetoprotein and human lymphocyte subpopulations. J Immunol 1977; 118:405-8.
4. Murgita RA, Anderson LC, Sherman MS, Bentwich H, Wigzell H. Effects of human alpha-fetoprotein in human B and T lymphocyte proliferation *in vitro*. Clin Exp Immunol 1978; 33:347-56.
5. Chism SE, Order SE, Hollman S. Tumor-fetal antigens in Hodgkin's disease: an immunoelectrophoretic analysis. Am J Roentgenol Radium Ther Nucl Med 1973; 117:5-11.
6. Moroz C, Giler S, Kupfer B, Urca I. Lymphocytes bearing surface ferritin in patients with Hodgkin's disease and breast cancer. N Engl J Med 1977; 296:1172-3.
7. Mukherji B. Hirschaut Y. Evidence for fetal antigen in human sarcoma. Science 1973; 181:440-2.
8. Pathouli Ch, Karvountzis G, Stergiou A, Hadziyannis S, Komninos Z. AFP-positive lymphocytes in lymphoproliferative disorders, hypogammaglobulinemias and liver diseases. Abstracts of the Meeting on Immunological Aspects of Experimental and Clinical Cancer, October 28 – November 1, 1979, Tel-Aviv, p. 122.
9. Bentwich S, Cohen I, Sulitzeanu D, Iszak G, Weiss DW. Antigenicity of lymphocytes from chronic lymphocytic leukemia (CLL) patients and from normal newborns. Proceedings of the American Association of Cancer Research. 1970; 2:7.
10. Anderson LC, Wasastjerna C, Calemberg CG. Different surface glycoprotein patterns on human T-, B-, and leukemic-lymphocytes. Int J Cancer 1976; 17:40-6.
11. Rundles WR. Chronic lymphocytic leukemia. In: Williams WJ, Beutler E, Erslev AJ, Rundles RW, eds, Hematology. 2nd ed. New York: McGraw Hill, 1977: 1004.
12. Komlos L, Halbrecht I, Shachor J, Djaldetti M. The effect of sera from chronic lymphocytic leukemia patients on normal mitogen-stimulated lymphocytes. A possible correlation with the patients' T and B lymphocytes. Exp Hematol 1978; 6: 595-600.
13. Ray KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternak BS. Clinical staging of chronic lymphocytic leukemia. Blood 1976; 46:219-34.
14. Gupta S, Good RA. Subpopulations of human T lymphocytes. I. Studies in immunodeficient patients. Clin Exp Immunol 1977; 30:222-8.
15. Boyum A. Separation of leukoyctes from blood and bone marrow. Scand J Clin Lab Invest 1968; Suppl 97:31-5.
16. Bentwich A, Douglas SD, Siegal FP, Kunkel HG. Human-lymphocyte-sheep erythrocyte formation: some characteristics of the interaction. Clin Immunol Immunopathol 1973; 1:511-22.
17. Speckart SF, Boldt DH, Ryerson KL. Cell surface changes in transformed human lymphocytes. I. Con A and E-PHA induced unique changes in surface topography. Exp Cell Res 1978; 111:385-95.
18. Rhodes J. Modulation of macrophage Fc receptor expression in vitro by insulin and cyclic nucleotides. Nature 1975; 257:597-9.