Changes of Adult T Cell Leukemia Cell Surface Antigens at Relapse or at Exacerbation Phase After Chemotherapy Defined by Use of Monoclonal Antibodies

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Surface phenotypes of leukemic cells from six patients with adult T cell leukemia (ATL) were analyzed by the use of monoclonal antibodies, both at the time of initial diagnosis and at either relapse or exacerbation phase after chemotherapy. Changes of cell surface antigens were observed in four of the six cases. The majority of the leukemic cells of these patients were reactive with anti-Leu-1 and anti-Leu-3a, but unreactive with anti-Leu-2a and MAS 036c monoclonal antibodies at the time of initial diagnosis, indicating that ATL cells are of peripheral inducer/helper T cell origin. In three cases, the Leu-1 antigen disappeared at relapse or at exacerbation phase, and in one of these cases, a small percentage of ATL cells became reactive with MAS 036c, which identifies cortical thymocyte antigen. ATL cells of one other case did not have Leu-1 antigen from the start, but gained Leu-2a antigen at exacerbation phase and became double-labeled cells (Leu-2a$^+$,3a$^+$), which is known to be a feature of thymocytes. Thus, it appeared that ATL cells sometimes change their surface phenotype to that of an earlier stage of T cell differentiation at relapse or at exacerbation phase. Chronic myelocytic leukemia (CML) cells also usually change to immature cells at blastic crisis involving morphological change. However, this morphological change was not so prominent in the ATL cases studied, except one, in which typical ATL cells with nuclear indentation changed to large immature cells with basophilic cytoplasm at relapse.

ADULT T CELL LEUKEMIA (ATL) is a T cell malignancy that frequently occurs in adults who are natives of the southwestern districts of Japan and has characteristic clinical and hematologic features.[1-3] This disease is becoming the object of worldwide attention because of its close relationship to the newly defined type C retrovirus.[4] We have shown that ATL cells have potent suppressor activity on pokeweed mitogen (PWM) induced normal B cell differentiation to immunoglobulin-producing cells, though these cells bear helper T cell antigen (Leu-3a).[5]

The prognosis of ATL is quite poor, and the median survival duration is only six to ten months.[2,3] The response of ATL cells to antineoplastic agents, like vincristine and adriamycin, is fairly good at first. However, these therapies lead to severe immunosuppressive states and cause fatal bacterial, fungal, and *Pneumocystis carinii* infections.[3] If the patients escape such infections and obtain partial remission, ATL cells may abruptly increase in number, having developed resistance to chemotherapy. We determined the surface antigens of ATL cells both at the time of initial diagnosis and at either relapse or exacerbation phase by use of monoclonal anti-T cell antibodies and compared the two sets of results.

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MATERIALS AND METHODS

Subjects

Six cases of ATL, in which surface phenotypes of leukemic cells at the time of initial diagnosis and at either relapse or exacerbation phase after chemotherapy could be determined, were selected for study. The diagnosis of ATL was based on the appearance of characteristic leukemic cells and by clinical and hematologic examination.[1-3] No cases of T cell acute lymphoblastic leukemia or lymphoma were included in this study. These patients were treated with vincristine (VCR) and prednisone (P), with or without cyclophosphamide (CY), adriamycin (ADR), and methotrexate (MTX).

Methods

A quantity of heparinized peripheral blood was obtained from the patients, and the surface phenotypes of ATL-enriched cells were studied according to the following method.[5] Mononuclear cells were separated by Ficoll-Conray density gradient centrifugation and were passed through a nylon wool column to avoid contamination with B cells and monocytes; ATL-enriched cells were obtained. A quantity of 10^6 ATL-enriched cells was incubated at 4 °C for 45 minutes with 0.1 mL of an appropriate dilution of monoclonal antibodies [anti-Leu-1: antibody against whole T cell antigen; anti-Leu-2a: antibody against cytotoxic/suppressor T cell antigen; anti-Leu-3a: antibody against inducer/helper T cell antigen; anti-HLA-DR: antibody against HLA-DR antigen (Becton Dickinson Monoclonal Antibody Center, Sunnyvale, Calif); and MAS 036c: antibody against thymocyte antigen (Sera-Labs, England)]. After washing three times with phosphate-buffered saline (PBS), the cells were treated with 0.05 mL of a 5% solution of fluorescein-conjugated $F(ab')_2$ fragment rabbit anti-mouse IgG (R/M FITC, Cappel Labs, Cochranville, Pa) at 4 °C for 45 minutes. The cells were washed three times with PBS and were processed on a fluorescence-activated cell sorter IV (FACS IV, Becton Dickinson Electronics Labs, Mountain View, Calif) or examined by fluorescence microscope.

RESULTS

Hematologic data of the six cases of ATL examined at the time of initial diagnosis and at either relapse or exacerbation phase are presented in Table 1, along with therapy regimens. Their ages ranged from 34 to 83 years; one patient was female and five were male. The WBC count and the percentage of ATL cells at the time of initial diagnosis ranged from 9,000/μL to 156,050/μL and 22% to 91%, respectively. The anemia and thrombocytopenia frequently involved in acute leukemia were not found at the time of initial diagnosis. Cases 4, 5, and 6 were treated with VP (VCR and P) and cases 1, 2, and 3 were treated chiefly with CVP-A (CY, VCR, P, and ADR). Cases 2, 3, and 4 obtained a partial remission—a state of no increase at ATL cells, though a small number of ATL cells remains in the peripheral blood after having stopped therapy. Relapse occurred after partial remission durations of from seven to ten months in these cases. In cases 1, 5, and 6, ATL cells decreased for a short time as a result of chemotherapy, but increased abruptly after having developed resistance to antineoplastic drugs; the patients died shortly thereafter. The WBC counts and the percentages of ATL cells at relapse or at exacerbation phase increased to the range of 23,400/μL to 215,000/μL and 50% to 98%, respectively. The morphological changes of ATL cells were not so prominent, and large cells slightly increased in number in cases 1, 3, 4, 5, and 6. In case 2, initial mature-type ATL cells with nuclear indentation changed to large cells with basophilic cytoplasm at relapse. Leukemic cells of cases 1, 2, and 5 at the time of initial diagnosis and at either relapse or exacerbation phase are shown in Fig 1.

The surface phenotypes of ATL-enriched cells at the time of initial diagnosis and at either relapse or exacerbation phase are presented in Table 2. Surface antigens changed in four of the six cases. Leu-1 antigen-bearing cells disappeared or decreased greatly in number in cases 2, 5, and 6, and interestingly, MAS 036c reactive cells appeared in case 6. As the sum of the percentages of cells bearing Leu-2a and Leu-3a exceeds 100% in this case, the appearance of double-labeled cells (Leu-2a$^+$,3a$^+$), which is characteristic of thymocytes, was supposed. In case 1, Leu-1 antigen was not present at the time of initial diagnosis, in spite of positive reaction against anti–Leu-3a, and the ATL cells gained Leu-2a antigen at exacerbation phase, and almost all cells became double-labeled cells, though these cells were unreactive with MAS 036c. Change of surface phenotype was not observed in cases 3 and 4, but there was a trend toward a decrease in the number of cells bearing Leu-2a and an increase of those with Leu-3a at relapse. This was believed to indicate that the percentage of ATL cells against normal T cells in peripheral blood had increased. The reactivity against anti-HLA-DR antibody did not show any particular trend. Clinical course, therapy regimens, and surface phenotypes of ATL-enriched cells at the time of initial diagnosis and at either relapse or exacerbation phase of cases 1 and 2 are shown in Figs 2 and 3, respectively.

DISCUSSION

Human T cell malignancies have been extensively investigated for cell surface antigens due to the recent advances in monoclonal antibody study. Inducer/helper T cell origin (OKT4$^+$/T8$^-$) of almost all cases of Sézary's syndrome (SS) and mycosis fungoides (MF) and the majority of cases of T lymphoma (T-L) and T prolymphocytic leukemia (T-PLL) has been reported, but a number of cases with T-L and T-PLL have both inducer/helper and killer/suppressor T cell antigens (OKT4$^+$/T8$^+$).[6-8] T cell antigens in cases of T chronic lymphocytic leukemia (T-CLL) appear to be

Table 1. Hematologic Data and Therapy Regimens

Case No.	Sex	Age (Years)			Hb (g/dL)	Platelet (Cells/μL)	WBC (Cells/μL)	ATL Cells (%)	Therapy
1	M	55	Initial	4 mo*	16.8	136,000	14,600	58	CVP-AM†
			Exacerbation		11.8	134,000	63,700	91	
2	F	61	Initial	8 mo*	11.0	173,000	9,000	59	CVP-A
			Relapse		13.7	109,000	23,400	50	
3	F	46	Initial	7 mo*	15.5	280,000	23,700	22	CVP-A
			Relapse		13.3	185,000	29,200	93	
4	F	64	Initial	10 mo*	10.0	360,000	49,000	63	VP
			Relapse		6.2	390,000	56,550	74	
5	F	83	Initial	3 mo*	10.3	90,000	156,050	91	VP
			Exacerbation		8.1	110,000	118,150	98	
6	F	34	Initial	9 d*	10.7	400,000	49,600	34	VP
			Exacerbation		14.0	ND	215,000	90	

*Period from the time of initial diagnosis to either relapse or exacerbation phase.

†C, cyclophosphamide; V, vincristine; P, prednisone; A, adriamycin; M, methotrexate.

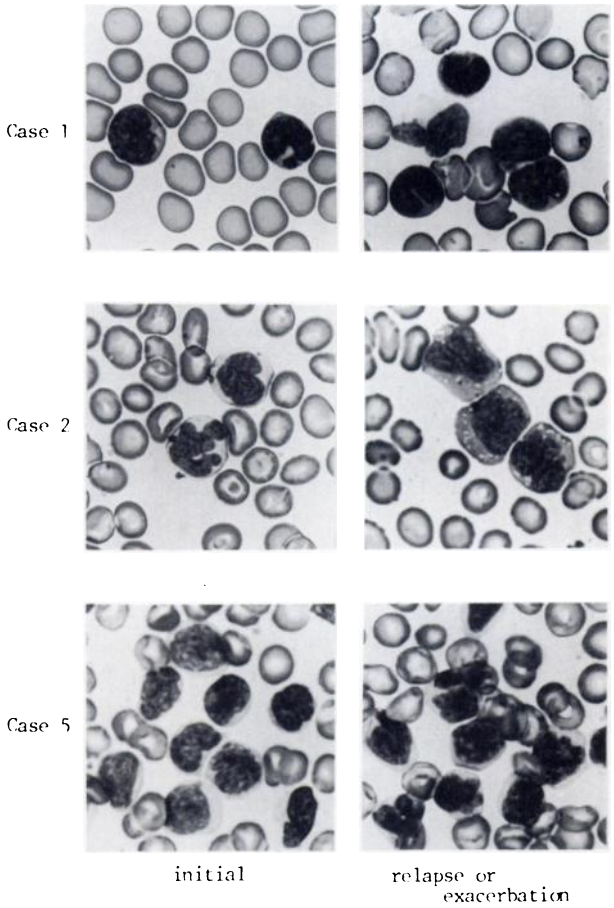

Fig 1. Leukemic cells of cases 1, 2, and 5 at the time of initial diagnosis and at either relapse or exacerbation phase. Morphological changes are not prominent in cases 1 and 5, and characteristic ATL cells that have cleaved and lobulated nuclei can be seen both at the time of intial diagnosis and at exacerbation phase. In case 2, initial characteristic ATL cells changed to large cells, which have vacuoles in basophilic cytoplasm and whose nuclear chromatin are fine.

divided into inducer/helper phenotype ($OKT4^+/T8^-$) and killer/suppressor phenotype ($OKT4^-/T8^+$).[6] We have reported that ATL cells have inducer/helper T cell antigen (Leu-3a), as in cutaneous T cell lymphoma (SS and MF).[5]

The present study revealed that the surface phenotypes of ATL cells defined at the time of initial diagnosis sometimes changes at either relapse or exacerbation phase. Changes of surface phenotypes occurred in four of the six examined cases: loss of Leu-1 antigen in three cases ($Leu\text{-}1^-,3a^+$) and gain of Leu-2a antigen in one case ($Leu\text{-}1^-,2a^+,3a^+$). The presence of $Leu\text{-}1^-,3a^+$ phenotype has been reported also in a number of cases with cutaneous T cell lymphoma[9] and acute lymphoblastic leukemia.[10] This may indicate the presence of such a phenotype in a differentiation stage of normal T cell lineage. Homologues of human Leu-1 antigen and mouse Lyt-1 antigen have been reported, and they increase in density as thymocytes mature. In other words, medullary thymocytes (mature thymocytes) and peripheral T cells have abundant Leu-1 antigen on their cell surface, but cortical thymocytes (immature thymocytes) have little.[11,12] The loss of Leu-1 antigen from ATL cells at relapse or at exacerbation phase may support the hypothesis that ATL cells have transformed into immature cells and proliferated. The appearance of cells reactive with MAS 036c in one case and of double-labeled cells ($Leu\text{-}2a^+,3a^+$) in two cases further supports this hypothesis. These findings suggest that blastic crisis-like phenomena, as seen in chronic myelocytic leukemia, can also occur in ATL.

Changes of surface antigens at relapse have been reported also in four of five cases with T cell lymphoblastic lymphoma.[13] The malignant cells appeared to be arrested at an earlier stage of differentiation in two cases and at a later stage in the two other cases. Change of surface antigen accompanying the clonal evolution of chromosomes has been reported in a case

Table 2. Surface Phenotypes of ATL-Enriched Cells

Case No.		ATL-Enriched Cells Reactive With Monoclonal Antibodies (%)				
		Leu-1	Leu-2a	Leu-3a	HLA-DR	MAS 036c
1	Initial	3.6	12.8	81.5	8.0	0.0
	Exacerbation	1.4	83.8	98.0	2.1	0.0
2	Initial	98.1	6.4	93.9	43.1	0.0
	Relapse	1.1	4.5	94.6	72.5	0.0
3	Initial	69.9	25.3	52.1	20.0	0.0
	Relapse	93.5	2.1	89.9	1.5	0.0
4	Initial	90.0	6.1	82.4	3.3	0.0
	Relapse	98.7	2.1	97.7	ND	ND
5	Initial	40.1	0.0	87.1	60.7	0.0
	Exacerbation	7.6	0.7	97.2	83.2	0.0
6	Initial	85.0	6.4	97.2	60.0	0.0
	Exacerbation	6.7	14.9	98.8	46.8	9.0

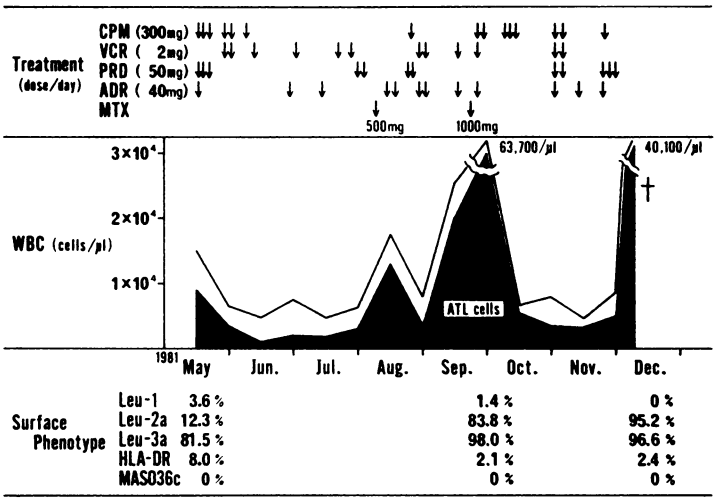

Fig 2. Clinical course, therapy regimen, and surface phenotypes of ATL-enriched cells at the time of initial diagnosis and at exacerbation phases of case 1. The patient was a 55-year-old male who became aware of generalized lymphadenopathy from the beginning of May 1981. He was referred to the Department of Internal Medicine of Nagasaki City Hospital on May 13. The WBC count and the percentage of ATL cells at the time of initial diagnosis were 14,600/μL and 58%, respectively. The reactivity of ATL-enriched cells with monoclonal antibodies was Leu-1, 3.6%, Leu-2a, 12.3%, Leu-3a, 81.5%, HLA-DR, 8.0%, and MAS 036c, 0%. He was treated with CVP-A (CY, VCR, P, and ADR), and ATL cells decreased in number but were not eliminated from the peripheral blood. WBC and the percentage of ATL cells increased to 17,600/μL and 81%, respectively, in mid-August, although chemotherapy had been continued. Treatment with 500 mg MTX was added, but the effect was brief, and WBC and the percentage of ATL cells increased to 63,700/μL and 91% in late-September. The reactivity of ATL-enriched cells with anti-Leu-2a monoclonal antibody increased to 83.8%, and it was assumed that ATL cells became double-labeled cells (Leu-2a$^+$,3a$^+$). The dose of MTX was increased to 1,000 mg, and this was effective and a temporary lull state was obtained. However, WBC and the percentage of ATL cells increased abruptly again from the beginning of December and reached 40,100/μL and 88%. He developed *Pneumocystis carinii* pneumonia and died on Dec 7, 1981. The reactivity of ATL-enriched cells with anti-Leu-2a monoclonal antibody before death increased further to 95.2%.

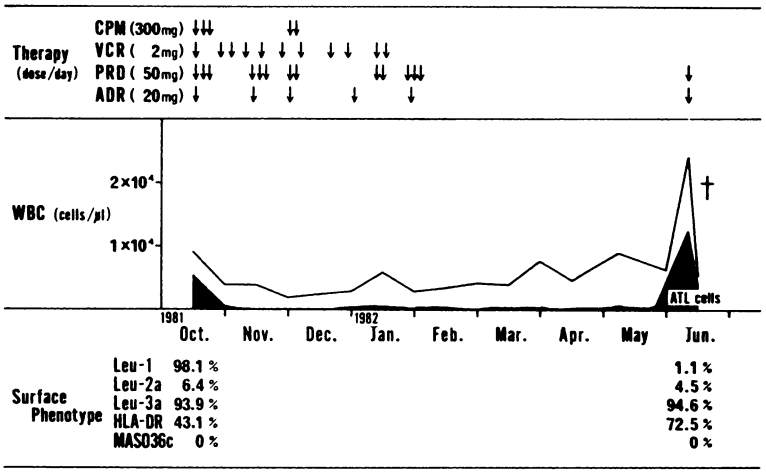

Fig 3. Clinical course, therapy regimen, and surface phenotypes of ATL-enriched cells at the time of initial diagnosis and at relapse of case 2. The patient was a 61-year-old female who had a sore throat and swelling of the right tonsil from August 1981. She visited the Department of Internal Medicine of Nagasaki City Hospital on Oct 15, 1981, when the WBC count and the percentage of ATL cells were 9,000/μL and 59%, respectively. The reactivity of ATL-enriched cells with monoclonal antibodies was Leu-1, 98.1%, Leu-2a, 6.4%, Leu-3a, 93.9%, HLA-DR, 43.1%, and MAS 036c, 0%. She was treated with CVP-A, and ATL cells decreased rapidly to a very low percentage. Chemotherapy was continued until late January 1982, and she was discharged in a condition of partial remission. She was followed as an out-patient without chemotherapy, but ATL cells did not increase for about four months. She noticed dyspnea from late May, and WBC count and the percentage of ATL cells suddenly increased and reached 23,400/μL and 50% on June 6. The reactivity of ATL-enriched cells with anti-Leu-1 antibody decreased greatly to 1.1%. She was treated with P and ADR, but died on June 11, 1982, from massive infiltration of ATL cells into both lungs.

with prolymphocytic leukemia of B cell origin ($\kappa\mu \rightarrow \kappa\gamma$).[14] We also have found the occurrence of clonal evolution of chromosomes at relapse in ATL cases other than those listed here (data not shown). We believe that crisis-like phenomena occur in lymphoid malignancies as well as in chronic myelocytic leukemia.

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REFERENCES

1. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H: Adult T-cell leukemia: Clinical and hematologic features of 16 cases. Blood 50:481, 1977

2. Ichimaru M, Kinoshita K, Kamihira S, Ikeda S, Yamada Y, Amagasaki T: T-cell malignant lymphoma in Nagasaki district and its problems. Jpn J Clin Oncol 9(suppl 1):337, 1979

3. Kinoshita K, Kamihira S, Ikeda S, Yamada Y, Muta T, Kitamura T, Ichimaru M, Matsuo T: Clinical, hematological and pathological features of leukemic T cell lymphoma. Cancer 50:1554, 1982

4. Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto T, Kinoshita K, Shirakawa S, Miyoshi I: Adult T-cell leukemia: Antigen in an ATL cell line and detection of antibodies to the antigen in human sera. Proc Natl Acad Sci USA 78:6476, 1981

5. Yamada Y: Phenotypic and functional analysis of leukemic cells from 16 patients with adult T-cell leukemia/lymphoma. Blood 61:192, 1983

6. Greaves MF, Rao J, Hariri G, Verbi W, Catovsky D, Kung P, Goldstein G: Phenotypic heterogeneity and cellular origins of T cell malignancies. Leuk Res 5:281, 1981

7. Knowles DM, Halper JP, Machin GA, Byeff P, Mertelsman R, Chess L: Phenotypic heterogeneity of human T-cell malignancies: Demonstration by monoclonal antibodies and cytochemical markers. Am J Hematol 12:233, 1982

8. Foon KA, Schroff RW, Gale RP: Surface markers on leukemia and lymphoma cells: Recent advances. Blood 60:1, 1982

9. Wood GS, Deneau DG, Miller RA, Levy R, Hoppe RT, Warnke RA: Subtypes of cutaneous T-cell lymphoma defined by expression of Leu-1 and Ia. Blood 59:876, 1982

10. Koziner B, Gebhard D, Denny T, McKenzie S, Clarkson BD, Miller DA, Evans RL: Analysis of T-cell differentiation antigens in acute lymphatic leukemia using monoclonal antibodies. Blood 60:752, 1982

11. Ledbetter JA, Evans RL, Lipinski M, Cunningham-Rundles C, Good RA, Herzenberg LA: Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. J Exp Med 153:310, 1981

12. Engleman EG, Warnke R, Fox RI, Dilley J, Benike CJ, Levy R: Studies of a human T lymphocyte antigen recognized by a monoclonal antibody. Proc Natl Acad Sci USA 78:1791, 1981

13. Bernard A, Raynal B, Lemerle J, Boumsell L: Changes in surface antigens on malignant T cells from lymphoblastic lymphoma at relapse: An appraisal with monoclonal antibodies and microfluorometry. Blood 59:809, 1982

14. Sadamori N, Han T, Minowada J, Bloom ML, Henderson ES, Sandberg AA: Possible specific chromosome change in prolymphocytic leukemia. Blood 62:729, 1983