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Abstract

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PRESERVED CELLULAR IMMUNITY IN SMOLDERING ACUTE LEUKEMIA

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Abstract. "Smoldering acute leukemia", a variant of acute myelogenous leukemia, has been recognized with frequent incidence in recent years. This is characterized by benign clinical course, poor physical findings, leukopenia and mild anemia in the peripheral blood, and apparent infiltration of abnormal myeloblasts in the bone marrow. Immunological studies of the host defence mechanism were made, because the pathogenesis of its "smoldering" course has never been well understood. Nine cases, seen during last 2 years, were investigated for immunological profile, especially the cellular immunity. Purified protein derivative (PPD) skin test, i.e., tuberculin test, was found to be positive in 8 of 9 cases (88.9%). Dinitrochlorobenzene (DNCB) sensitization test showed to be positive in 4 of 6 cases examined (66.9%). Peripheral lymphocyte blastogenesis by stimulating with phytohemagglutinin (PHA) was evaluated using the smear counting method. The blastoid lymphocyte ratio was 55% at the median value (range: 31-68%), compared with 63% in normal young control (age: 25-32) and 41% in normal aged control (age: 60-75). In this report, the cellular immunity in smoldering acute leukemia was proved to be preserved at the normal level and to be more competent than that in aged group. The preserved cellular immunity is considered to explain the phenomenon of "smoldering", in other words, the exacerbating proliferation of leukemic cells is suppressed by immuno-surveillance system.

"Smoldering acute leukemia" was designated first to be a variant of acute myelogenous leukemia by Rheingold *et al.* in 1963 (1).

This type of leukemia has been recognized with frequent incidence in recent years, in proportion to the increasing ratio of acute leukemia among aged people (2) (3) (4).

The clinical pictures are characterized as follows: (i) A variant of acute myelogenous leukemia, mostly seen among aged patients. (ii) Insidious onset and prolonged course without specific chemotherapy. (iii) No life-threatening acute infection and bleeding and no striking hepatosplenomegaly and lymphadenopathy. (iv) Mild anemia and leukopenia with a few blasts in the peripheral blood. And, (v) diagnostic bone marrow with infiltration of atypical myeloblasts, "wild looking" and with prominent nucleoli (1) (3) (5).

The bone marrow of smoldering acute leukemia may be hypocellular, normocellular or hypercellular, and the number of blast cells may vary from somewhere between 5 to 10 percent to almost complete infiltration. Most of cases with hypoplastic leukemia may survive longer than the typical leukemia, and by careful retrospective review, smoldering acute leukemia can be diagnosed (1) (3).

Although the clinical entity of smoldering acute leukemia can be differentiated from typical acute leukemia, the pathogenesis of "smoldering" course has never been well understood.

Recently there have been accumulated knowledges about the cellular immunity in malignant diseases, including cancers and malignant lymphomas. And the cellular immunity for host defence mechanism has been found to play an important role in malignant diseases. In typical acute leukemia, several opinions concerning changes of immuno-competence which depends on the severity of disease have been discussed by the study of delayed hypersensitivity skin tests.

Because the immunological studies on smoldering acute leukemia has never been reported previously, the following clinical investigations were made on 9 patients.

In this report, the analysis of the preserved competence of cellular immunity in smoldering acute leukemia was demonstrated by means of delayed hypersensitivity skin tests and of the peripheral lymphocyte blastogenesis by stimulating with phytohemagglutinin. And, the role of cellular immunity on the host-tumor relationship in the condition of "smoldering" state was discussed.

MATERIALS AND METHODS

Patients: Nine patients had been encountered from September 1973 to December 1975; all cases were diagnosed as acute myelogenous leukemia. "Smoldering" acute leukemia was diagnosed retrospectively with the aids of criteria, described before.

Healthy controls consisted of the young group (age: 25-32) and the aged group (age: 60-75). Thirty patients with acute non-lymphocytic leukemia (ANLL) (aged: 13-62), including acute myelogenous leukemia (AML), acute promyelocytic leukemia (APL), monocytic leukemia (MoL), and erythroleukemia (ErL), and 14 cases of acute lymphocytic leukemia (ALL) (age: 15-66) were also chosen as controls.

Humoral immunological tests: Serological tests, including CRP, RA, ASLO, Wasserman reaction, Australian antigen and Coombs' test, were done in routine methods. Serum protein electrophoresis and immunoglobulin were examined also in routine methods, described elsewhere.

Purified protein derivative (PPD) skin test, i.e. tuberculin test: PPD (intermediate strength) used was a product of Nippon BCG Company. 0.1 ml was injected

intradermally and the reactivity was measured after 48 hr. Then, this skin test was judged as follows: (−): no reaction. (±): 5-9 mm of erythema with induration. (+): more than 10 mm of erythema with induration. (++): more than 10 mm of induration with double erythema.

Dinitrochlorobenzene (DNCB) sensitization test: DNCB (Ishizu Pharmaceutical Company) was dissolved with acetone. 0.1 ml of DNCB solution (2000 μg) was sprayed topically on the shoulder, allowed to dry and covered with cotton bandage for sensitization. Two weeks later, the challenge test was done on the forearm with DNCB solutions of 2 different concentrations (50 μg and 100 μg). Subsequently, this sensitization test was judged as follows. (6) (7): (−): no reaction. (+): erythema. (++): erythema with induration. (+++): erythema with induration and vesiculation.

Lymphocyte blastogenesis by stimulating with phytohemagglutinin (PHA): Venous blood (3-12 ml) was drawn and lymphocytes were separated using a Lymphoprep (Nyegaard and Co., Norway).

Collected lymphocytes were washed twice with the culture medium, RPMI 1640. 2×10^6 of lymphocytes were suspended in the culture medium, consisting of 2 ml RPMI1640 supplemented with 15% fetal calf serum. Phytohemagglutinin-P (Difco Laboratories, U.S.A.), dissolved with RPMI1640, was added in this culture tube at the final concentration of 5 μl per ml. This culture tube was placed for 72 hr in an incubator with 5% CO_2 atmosphere.

Then, the cultured lymphocytes were sedimented and collected to prepare the smear on slide glasses. Smear was stained with May-Grünwald-Giemsa solution. Blastoid lymphocytes were counted on 2 slide preparations by 2 hematologists for objective observation. The criteria of blastoid lymphocyte were (i) increased cell size (12-20 μm) and abnormal cell shape, (ii) increased nucleous size with fine chromatin structure, (iii) a few apparent nucleoli, (iv) wide basophilic cytoplasm, and (v) sometimes reticulum cell-like blastoid cells. The average value in each was expressed as percentage for the ratio of lymphocyte blastogenesis.

RESULTS

Smoldering acute leukemia belonged to "myelogenous" type in all cases. Males were more frequent than females (male: female = 7 : 2). Ages ranged between 52 and 76, but 7 of 9 cases were more than 60 years old. (Table 1)

In clinical findings, slight hepatomegaly was seen in 4 cases, one case had high fever due to urinary tract infection and one had mild petechiae. In regard to the clinical course, the longest duration of survival from the time of diagnosis was 3 years and 9 months, and most of the cases survived one to 3 years with supportive therapy by blood transfusion.

The peripheral blood showed 0 to 5% of myeloblasts with leukopenia commonly found. Case 2, who had 6400 of leukocyte count by the time of diagnosis, became leukopenic later during chronic course. In the bone marrow, myeloblasts occupied 7.2 to 51.6%, and by careful observation the myeloblast

was characterized by an atypical immature cell with prominent nucleoli. (Table 2)

Table 1 Cases of smoldering acute leukemia: Clinical findings on admission

Case	patient	sex	age	type	fever	bleeding tendency	hepato-megaly	spleno-megaly	lymph-adenopathy
1.	T.Y.	F	76	AML	(+)	(−)	(−)	(−)	(−)
2.	Y.H.	M	72	AML	(−)	(−)	(−)	(−)	(−)
3.	M.T.	F	66	AML	(−)	(−)	(−)	(−)	(−)
4.	T.M.	M	66	AML	(−)	(−)	(+)	(−)	(−)
5.	N.K.	M	66	AML	(−)	(−)	(+)	(−)	(−)
6.	K.M.	M	64	AML	(−)	(+)	(−)	(−)	(−)
7.	M.M.	M	52	AML	(−)	(−)	(+)	(−)	(−)
8.	A.K.	M	47	AML	(−)	(−)	(+)	(−)	(−)
9.	T.K.	M	71	AML	(−)	(−)	(−)	(−)	(−)

Table 2 Hematological data on the time of diagnosis

case	Peripheral Blood				Differ.	Bone Marrow			
	Hb. (mg/dl)	RBC ($\times 10^4$)	WBC	Plat. ($\times 10^4$)	Myelo-blast (%)	NCC ($\times 10^4$)	Myelo-blast (%)	Promyelo-cyte (%)	Eryth-roid (%)
1.	7.5	240	800	26.0	1	9.0	51.6	1.0	21.8
2.	8.5	225	6400	11.8	1	20.6	7.2	10.8	28.6
3.	5.0	174	2800	1.5	1	—	11.4	9.6	38.2
4.	5.0	163	1400	4.1	0	—	9.4	5.6	42.6
5.	3.7	110	1700	8.6	1	6.8	26.0	16.8	1.2
6.	7.7	210	2900	0.2	5	7.5	8.4	19.0	50.4
7.	6.2	205	600	1.2	3	0.3	8.8	8.4	38.8
8.	5.0	103	1900	0.9	2	7.3	43.2	4.8	18.0
9.	8.3	200	700	6.4	2	7.0	32.4	2.8	15.2

Serological tests revealed no particular tendency to any test. The positive CRP was commonly seen in leukemia of acute phase. The electrophoretic pattern of serum protein showed the increased amount of γ-globulin fraction with generally increased IgG level. No monoclonal gammopathy was observed. (Table 3)

In cellular immunological profile, PPD test revealed the positive reactivity in 8 cases out of 9 (88.9%). DNCB test was positive in 4 out of 6 cases with erythema and induration. (Table 4)

Incidence of positive skin tests by PPD and DNCB in smoldering acute leukemia was compared with ANLL and ALL. Positive reactivity of 88.9%

by PPD test was significantly higher in comparison with 26.7% in ANLL and 35.7% in ALL. DNCB test showed the same tendency. (Table 5)

TABLE 3 EXAMINATIONS OF HUMORAL IMMUNOLOGICAL PROFILE

Case	CRP	RA	ASLO	Wa-R	Au-Ag	Coombs	Serum Protein (mg/dl)	γ-globulin fraction (%)	Immunoglobulin (mg/dl)		
									IgG	IgA	IgM
1.	(−)	(−)	60	(−)	(−)	(−)	7.3	27.8	1190	210	99
2.	(1+)	(−)	50	(−)	(−)	N.D.	6.9	23.3	3930	248	240
3.	(2+)	(−)	166	(−)	(−)	(−)	6.8	35.0	1780	210	132
4.	(3+)	(+)	100	(−)	(−)	N.D.	7.4	20.0	1880	258	351
5.	(2+)	(−)	100	(−)	(−)	(−)	8.1	40.3	3530	225	132
6.	(−)	(−)	12	(−)	N.D.	(−)	6.8	22.5	1650	174	78
7.	(4+)	(−)	N.D.	(−)	N.D.	N.D.	6.4	23.1	N.D.		
8.	(1+)	(−)	12	(+)	(−)	(−)	6.1	17.1	N.D.		
9.	(1+)	(−)	20	(−)	(−)	(−)	5.7	20.1	N.D.		

N.D.: Not done

TABLE 4 EXAMINATIONS OF CELLULAR IMMUNOLOGICAL PROFILE

Case	PPD test	DNCB test	Lymphocyte Blastogenesis (%)
1.	(+)	(−)	42
2.	(−)	N.D.	31
3.	(+)	(−)	68
4.	(+)	N.D.	42
5.	(+)	(+)	57
6.	(+)	(+)	65
7.	(+)	(+)	52
8.	(+)	(+)	61
9.	(+)	N.D.	N.D.

TABLE 5 INCIDENCE OF POSITIVE SKIN TESTS BY PPD AND DNCB IN SMOLDERING ACUTE LEUKEMIA, COMPARED WITH ACUTE LEUKEMIA

	Smoldering Acute Leukemia (AML) age: 52-76	ANLL (AML, APL, MoL, ErL) age: 13-62	ALL age: 15-66
PPD test ≧ 5 mm erythema and induration	88.9% (8/9 cases)	26.7% (8/30 cases)	35.7% (5/14 cases)
DNCB test erythema and induration or plus vesiculation	66.7% (4/6 cases)	33.3% (5/15 cases)	25.0% (1/4 cases)

The ratio of lymphocyte blastogeneisis ranged from 31% to 68% with median value of 55%. For the comparative evaluation, normal young and aged groups showed 63% and 41% in the median, respectively. (Fig. 1)

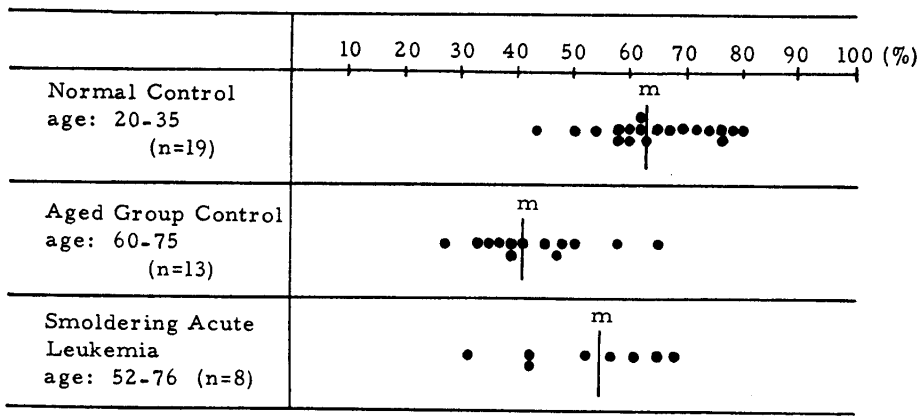

Fig. 1. Lymphocyte Blastogenesis by PHA stimulation (Smear Counting Method) in Smoldering Acute Leukemia, compared with Aged Group Control. (Ratio expressed in percentage) (m: median value)

DISCUSSION

In this paper, one aspect of immunological studies revealed the preserved cellular immunity in the patients with smoldering acute leukemia for the first time. The high incidence of positive reactivity by delayed hypersensitivity skin tests and the normal range of lymphocyte blastogenesis were demonstrated.

The immunity of smoldering acute leukemia should be discussed in two standpoints; one is the immunity of the aged people and the other is that of patients with acute myelogenous leukemia. The importance of the immunological tests in patients with malignant diseases was outlined with the special reference to T-lymphocyte function tests (8).

The relationship between the aging and the cellular immunity has been reported by several researchers. According to Koide *et al.*, the incidence of positive PPD reactivity in aged group ranging 60 to 79 was 58.7% with the control of 89.9% in younger group in Japanese population. Positive DNCB sensitization reactivity was 63.6% in aged group (9).

The ability to develop the delayed hypersensitivity has been known to become decreased according to aging.

According to Waldorf *et al.*, 68% of the aged more than 70 years old reacted to DNCB test in contrast to the normal responsiveness (94%) in 69 years old or less (10).

Nakayama *et al.* (11) demonstrated the tendency of decreasing ratio of blastogenesis in the peripheral lymphocytes by PHA stimulation in proportion to the aging. The significant correlation was apparently seen between the aging and the decreasing blastogenesis ratio as well as the correlation in the reactivity of delayed hypersensitivity.

These results suggest a close relationship of high incidence of malignancy and autoimmune disease in the aged people, who might have the decreased immuno-surveillance by T-lymphocyte function.

Immunological investigation on smoldering acute leukemia has never been reported before, although there are many reports about the immunity of acute leukemia, in relation to chemotherapy and immunotherapy. According to Dupuy *et al.* by studing several kinds of skin tests, delayed hypersensitivity in acute leukemia was shown to be slightly impaired before treatment and the depressed delayed hypersensitivity during chemotherapy was correlated with the severity of aplasia of the bone marrow (12). Hersh *et al.* emphasized that patients with normal delayed hypersensitivity had better prognosis than those with anergic reaction in the course of chemotherapy (13). Immunological study in acute leukemia has been more advanced in investigating the tumor-associated antigens, and the phenomenon of lymphocyte blastogenesis responding to the stimulation of leukemic cells has been well known. Gutterman *et al.* demonstrated that a favorable prognosis was correlated with a vigorous response of lymphocyte blastogenesis to autochthonous leukemic cells (14). Using BCG and radiated leukemic cells, the immunotherapy for acute leukemia was aimed to increase the host-defence immunity, by stimulating the immuno-surveillance system of T-cell function in order to eradicate residual leukemic cells (15) (16).

In this study, several new findings about immunological profile in smoldering acute leukemia were revealed. (i) Patients preserved the competent delayed hypersensitivity, in spite of old age, as high as young group. (ii) Peripheral lymphocyte reacted almost normally to the stimulation of PHA, even though peripheral blood was leukopenic and bone marrow was suppressed by the infiltrating leukemic cells.

Because of technical difficulty, tumor-associated antigen in smoldering acute leukemia has never been investigated yet. By T-cell function, however, host-defence mechanism to eliminate leukemic cells, which may be recognized as a not-self in antigenicity, is considered to explain the phenomenon of "smoldering"; in other words, clinical exacerbation due to proliferating leukemic cells is suppressed by immuno-surveillance system, and the prolongation of clinical course without any specific chemotherapy may be achieved, while leukemic cells are confined in the bone marrow.

Several studies in regard to the pathogenesis of smoldering acute leukemia

have been reported. Elevated muramidase levels and abnormal karyotypes by chromosome analysis were revealed by Knospe *et al.* (5). Kinoshita performed the kinetic study of leukemic blasts in smoldering acute leukemia by labeling cells with ^{3}H-thymidine, found that the labeling index was lower than that of the typical leukemia, and assumed that most of leukemic cells in smoldering acute leukemia were "dormant" cells (17).

One of the cases studied here had the long duration of survival up to 3 years and 9 months after the diagnosis. During its course, three episodes of pneumonia were considered retrospectively to contribute temporary improvement in hematological findings, and the bacterial infection was presumed to activate the non-specific immunity against leukemic cells in the host (18).

Recently clinical trial of neocarzinostatin, a new antitumor antibiotic, was found to be effective to reduce the infiltration of leukemic cells in the bone marrow in the cases of hypoplastic leukemia, including smoldering acute leukemia, because neocarzinostatin was less suppressive to the bone marrow hematopoiesis than other conventional anti-leukemic agents.

In order to find out a clue of leukemogenesis and to develop an effective treatment for acute leukemia, the enigmas concerning "smoldering" clinical course should be solved through further studies using immunological methods.

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