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Abstract

Forty-five patients with acute leukemia were compared on cellular immunity measures versus prognosis. The patients were treated according a multicombination therapy protocol. The purified protein derivative (PPD) test and dinitrochlorobenzene (DNCB) test on admission indicated low positive percentages. In acute non-lymphocytic leukemia (ANLL) patients, the 50% survival durations were 11 months in the PPD positive group and 6 months in the PPD negative group. In acute lymphocytic leukemia (ALL) patients, the 50% survival durations were 21 months in the PPD positive group and 13 months in the PPD negative group. Peripheral lymphocyte blastogenesis by phytohemagglutinin (PHA) stimulation was examined at various clinical stages. The stimulation indices were generally low, and no correlation was found between the PHA test and clinical stages. These cellular immunity measures appeared to reflect one aspect of the clinical condition in acute leukemia patients, and further studies are needed for predicting prognosis.

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THE RELATIONSHIP OF CELLULAR IMMUNITY TO PROGNOSIS IN ACUTE LEUKEMIA

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Abstract. Forty-five patients with acute leukemia were compared on cellular immunity measures versus prognosis. The patients were treated according a multicombination therapy protocol. The purified protein derivative (PPD) test and dinitrochlorobenzene (DNCB) test on admission indicated low positive percentages. In acute non-lymphocytic leukemia (ANLL) patients, the 50% survival durations were 11 months in the PPD positive group and 6 months in the PPD negative group. In acute lymphocytic leukemia (ALL) patients, the 50% survival durations were 21 months in the PPD positive group and 13 months in the PPD negative group. Peripheral lymphocyte blastogenesis by phytohemagglutinin (PHA) stimulation was examined at various clinical stages. The stimulation indices were generally low, and no correlation was found between the PHA test and clinical stages. These cellular immunity measures appeared to reflect one aspect of the clinical condition in acute leukemia patients, and further studies are needed for predicting prognosis.

Many studies have been conducted recently on the relationship between the clinical course in malignant diseases and cellular immunity. Most of these studies have been on the correlation between tumor regression and prognosis in solid tumors and Hodgkin's disease (1–5). Few reports have dealt with immunocompetence in acute leukemia (6–8), as its clinical manifestations change rapidly during various stages of chemotherapy and prognosis is markedly affected by sophisticated leukemia treatment. As reported previously (9), we have attained a high rate of complete remission and a longer survival duration using newly developed agents and a protocol of combined chemotherapy.

The purpose of this study was to examine changes in cellular immunity during the clinical course of acute leukemia by the delayed hypersensitivity skin tests and by the peripheral lymphocyte blastogenesis response to stimulation with phytohemagglutinin to predict prognosis (10).

MATERIALS AND METHODS

Forty-five patients treated from September 1973 to July 1975 in our clinic were evaluated. All patients were cared and followed-up until death or June 1976. The diagnoses were as follows: 31 patients with acute non-lymphocytic leukemias (ANLL), including 18 acute myelogenous leukemias (AML), 6 acute promyelocytic leukemias (APL) and 7 monocytic leukemias (MoL) and 14 patients with acute lymphocytic leukemias (ALL). ANLL and ALL were separated in the study because the clinical responses to chemotherapy and prognoses were apparently different in each group. Age and sex distributions are shown in Table 1.

TABLE 1. SEX AND AGE DISTRIBUTIONS OF PATIENTS OF THE ANLL AND ALL GROUPS

	Sex		Age range					
	Male	Female	15-19	20-29	30-39	40-49	50-59	60-66
ANLL	19	12	6	4	10	4	6	1
ALL	10	4	5	5	2	0	1	1

Therapy. Intensive anti-leukemic chemotherapy was administered in cycles of four consecutive days followed by a pause of 7 to 14 days until the remission criteria were satisfied. The treatment protocol of ANLL and ALL is summarized in Table 2. The details of treatment were previously reported (9).

TABLE 2. PROTOCOLS OF CHEMOTHERAPY IN ANLL AND ALL

I. Remission induction therapy

Combination used: NADP, NAMP, NDMP and NVMP.

(N) Neocarzinostatin—0.04-0.05 mg/kg, i.v. drip infusion (four days)
(A) Cytosine arabinoside—1.2-1.5 mg/kg, i.v. drip infusion (four days)
(D) Daunomycin—0.6-0.7 mg/kg, i.v. push (four days)
(M) 6-MP-riboside—5.0-5.5 mg/kg, i.v. push (four days)
(V) Vincristine—0.04-0.06 mg/kg, i.v. push (the first day)
(P) Prednisolone—0.8-1.0 mg/kg, i.v. drip infusion (four days)

II. Consolidation therapy

One course or two courses were administered of the same combination chemotherapy shown in I.

III. Cyclic maintenance therapy

Each drug was given in 4 week cycles.
6-MP—2 mg/kg/day, p.o.
Cyclophosphamide—2 mg/kg/day, p.o.
Methotrexate—0.05 mg/kg/day, p.o.

Purified protein derivative (PPD) test. Intermediate strength PPD (Nippon BCG Company, Tokyo) was used in the investigation. The sites of injections

were rotated every month to different regions of the forearm and upperarm to check the PPD reactivity. Criteria of positive reactivity were erythema and induration of more than 5mm mean diameter.

Dinitrochlorobenzene (DNCB) test. DNCB (Ishizu Pharmaceutical Company, Tokyo) was dissolved with acetone. This solution at 0.1 ml containing 2000 μg of DNCB was sprayed on the shoulder, allowed to dry and covered. Two weeks later challenge doses of 100 μg and 50 μg were applied on the forearm, and the reactions were interpreted as positive or negative 2 days later. Positive criteria were defined as induration and erythema with or without vesiculation (3).

Lymphocyte blastogenesis by phytohemagglutinin (PHA) stimulation. Venous blood was drawn from patients at various clinical conditions when the peripheral smears showed no leukemic cells in leukocyte differential counts. The method for evaluating blastogenesis was modified from the report of Pellegrino *et al.* (11), as shown in Fig. 1. Heparinized whole blood at 0.05 ml was suspended in 2ml of culture medium containing RPMI-1640 and 20% fetal calf serum.

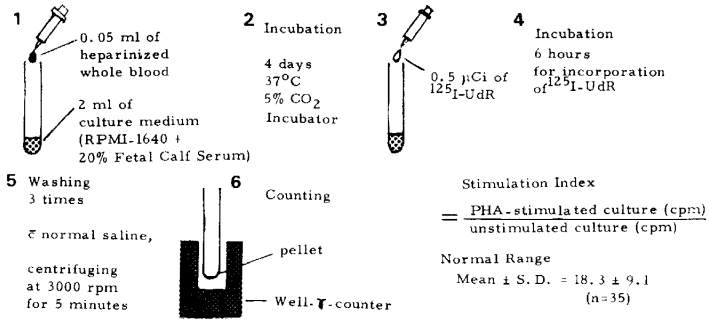

Fig. 1. Method of lymphocyte blastogenesis by stimulation with phytohemagglutinin (PHA).

PHA-P (Difco Laboratories, Detroit, U. S. A.) at 5 μl was added. The culture tubes were placed in an incubator with a 5% CO_2 atmosphere for 4 days. Six hours before termination of culture, 0.5 μCi of ^{125}I-UdR was added, and the mixture was incubated. The culture tubes were centrifuged and the medium was removed. The blood pellet was washed 3 times with normal saline. The counting was performed by a well-type gamma counter in triplicate samples, and the stimulation index was calculated.

RESULTS

All patients were treated according to the protocols for ANLL and ALL, and individual cases were not affected much by the different chemotherapeutic agents used in the follow-up study. In the ANLL group, the median durations of complete remission and survival were 4 months and 8 months, respectively. In the ALL group, the corresponding results were 6.5 months and 15 months, respectively (Table 3).

The results of the PPD test at different clinical stages are shown in Table 4. A correlation was not apparent between PPD reactivity and clinical stages. Both the ANLL and ALL group showed low ratios of positive reactivity. The DNCB test results also showed the same tendencies in the ANLL and ALL group (Table 5).

TABLE 3. MEDIAN DURATIONS OF COMPLETE REMISSION AND SURVIVAL IN ANLL AND ALL PATIENTS

Diagnosis	No. of patients	Median duration complete remission (mo.)	Median duration survival (mo.)
ANLL	31	4	8 (range 1-21)
ALL	14	6.5	15 (range 2-36)

TABLE 4. RESULTS OF PPD TEST IN DIFFERENT CLINICAL STAGES IN ANLL AND ALL PATIENTS

PPD Test	ANLL clinical stage			ALL clinical stage		
	Induction %	Complete remission %	Relapse %	Induction %	Complete remission %	Relapse %
Positive	35.5(8/30)	14.3(4/28)	10.0(2/20)	35.7(5/14)	46.2(6/13)	40.0(4/10)
Negative	64.5(22/30)	85.7(24/28)	90.0(18/20)	64.3(9/14)	53.8(7/13)	60.0(6/10)

Figures in parentheses represent the number of affected patient/total patient.

TABLE 5. RESULTS OF DNCB TEST IN ANLL AND ALL PATIENTS

DNCB Test	ANLL %	ALL %
Positive	35.7(5/14)	25.0(1/4)
Negative	64.3(9/14)	75.0(3/4)

Figures in parentheses represent the number of affected patient/total patient.

The prognosis was compared against the cellular immunity findings at the induction therapy stage. The survival curves of PPD positive and negative patients were drawn by actuarial method. In ANLL patients, the 50% median survival rate of the PPD positive group was 11 months after diagnosis and the PPD negative group, 6 months after diagnosis (Fig. 2). In ALL patients, the values were 21 months and 13 months, respectively, after diagnosis (Fig. 3). These results suggest a tendency for longer survival of patients reacting positively at the beginning of therapy in both the ANLL and ALL groups.

The results of lymphocyte blastogenesis in different clinical stages are shown in Fig. 4 and Fig. 5. The lymphocyte blastogenesis stimulation index in normal

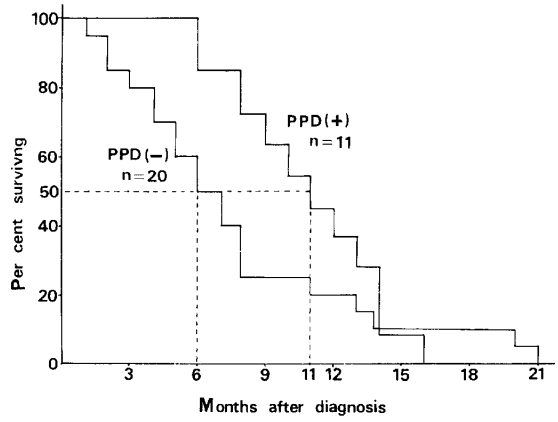

Fig. 2. Actuarial curve of ANLL patients against PPD reactivity. The PPD test was conducted on admission.

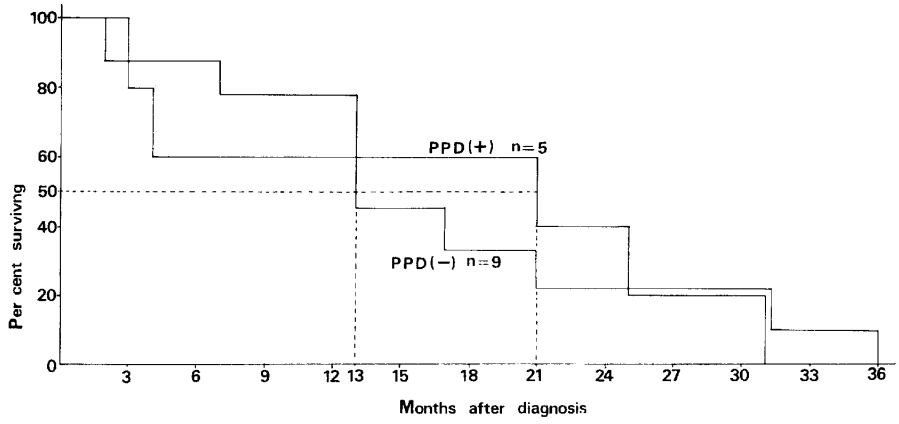

Fig. 3. Actuarial curve of ALL patients against PPD reactivity. The PPD test was conducted on admission.

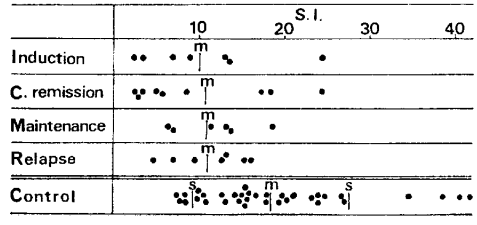

Fig. 4. PHA-induced lymphocyte blastogenesis in different stages of ANLL patients. The stimulation index was based on ^{125}I-UdR incorporation rate. S.I., stimulation index; m, mean; s, standard deviation; C. remission, complete remission; control, normal individuals.

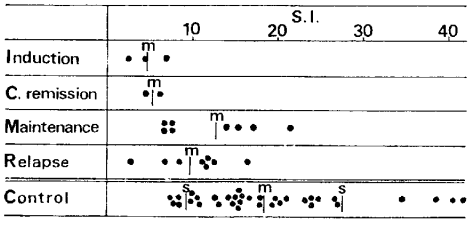

Fig. 5. PHA-induced lymphocyte blastogenesis in different stages of ALL patients. The stimulation index was based on ^{125}I-UdR incorporation rate.

controls was 18.3±9.1 (M ± S. D.). The stimulation indices during induction, complete remission, maintenance and relapse were 10.2, 10.7, 10.9 and 11.1, respectively, in ANLL; and the values were 4.4, 4.7, 12.8 and 9.9, respectively, in ALL. There was no correlation between lymphocyte blastogenesis and clinical stages. Furthermore, these results did not introduce evidence of immunocompetent recovery during stages of complete hematological remission.

DISCUSSION

This paper demonstrated that prognoses in acute leukemia and cellular immunity were closely related. It appears that prognosis may be more favorable when cellular immunity in the induction phase is sufficiently preserved.

The PPD test on admission may aid in predicting prognosis. In Japan the high incidence of PPD positive reactivity due to the widespread preventive use of BCG vaccination places more importance in the evaluation of the PPD recall antigen testing of cellular immunity compared with studies in Europe and the United States, where the positive PPD reactivity incidence is low in the general population.

The PHA-stimulated lymphocyte blastogenesis is considered to indicate T-lymphocyte function *in vitro* and has a close correlation with delayed cutaneous hypersensitivity (10). The microtechnique of using whole blood in the PHA test has the advantage of reflecting the immunological reactivity of the patient, as whole blood retains blood cells in natural proportions (11). For effective patient cellular immunity against malignant cells, not only lymphocyte function but total lymphocyte count is important. Therefore, the PHA test performed in this study was useful for measuring the whole body response by active T-lymphocytes.

The median value in the PHA test of the ANLL group was slightly higher than the value of the ALL group, and the cellular immunity of the ANLL group may be thought to be better preserved than that of ALL. However, the present study did not show a correlation between the PHA test and various clinical stages of ANLL and ALL.

Hersh and Oppenheim (1) reported that lymphocyte function in Hodgkin's disease was impaired and that the immunological response was related to the severity of the disease. The same conclusions were reported by several other researchers (2–4), although some data were inconsistent on the correlation between lymphocyte blastogenesis and disease severity (12, 13). In metastatic solid tumors and lung cancers, immunological studies have supported the concept of a correlation between cellular immunity and clinical course (5, 14).

Depuy *et al.* (6) showed that in acute leukemia the abolition of delayed hypersensitivity reactions occurring during drug-induced aplasias correlated with the severity of the aplasia and that the delayed hypersensitivity reactions were slightly impaired before treatment. Hersh *et al.* (7) reported that acute leukemia patients converting from immunoincompetence to immunocompetence during therapy achieved remission and that cell-mediated immunity correlated with the response to chemotherapy. Furthermore, the data of Green and Borella (15) in acute lymphocytic leukemia demonstrated immunological rebound after cessation of long-term chemotherapy.

In summary, the reviewed literature and our studies suggested that in acute leukemia prognosis and cellular immunity may be related. It may be further suggested that the cellular immunocomponent function by T-lymphocytes may help to eliminate residual leukemic cells after anti-leukemic drugs are administered to rapidly reduce the mass of leukemic cells. In this respect, T-lymphocyte function as a possible routine indicator of the clinical condition and prognosis in leukemia needs continued and further evaluation.

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