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NATURAL KILLER (NK) ACTIVITY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA*)

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ABSTRACT

The natural killer (NK) activity against K 562 target cells was explored in 20 children with acute lymphoblastic leukemia (ALL).

Three of 5 patients examined at the time of diagnosis had normal NK activity. Two patients, who had more than 80% circulating blasts had significantly low NK activity. The level of NK activity of these 5 patients was inversely proportional to the percentage of blast cells contaminating the effector cell populations. After initiating induction chemotherapy, the NK activity decreased in spite of disappearance of circulating blasts. After complete remission, their NK activity returned to normal levels, and was rather higher than that of onset of the disease.

NK activity of 15 children with ALL remaining in continuous complete remission was as high as that of healthy children and healthy adults in spite of intensive maintenance chemotherapy for 1/2 to 4 years. This was thought to be due to intermittent dose-schedule of the maintenance chemotherapy.

INTRODUCTION

The immunological status of children with acute lymphoblastic leukemia (ALL) has been evaluated by many investigators. At the time of diagnosis, it was reported to be deranged secondary to leukemic infiltration of the lymphoid tissues. They showed impaired delayed cutaneous hypersensitivity, impaired lymphocyte proliferative responses to phytohemagglutinin, and low serum immunoglobulin concentration[1-3].

In recent years, a marked improvement in the prognosis of childhood ALL has been attained. At Hiroshima University School of Medicine, the expected 5-year "leukemia-free" survival of children with ALL treated with the combination chemotherapy protocol, "HL-1", is 37%[4]. To cure children with ALL, it is mandatory to use a number of chemotherapeutic agents in combination for long periods (for 3 to 5 years). However, immunosuppressive effects of these chemotherapeutic agents may be hazardous for children with ALL, because they may also suppress antitumor immunological function. To attain true cure of leukemia, effective immune responsiveness may be of major importance[5-7].

Recently, natural killer (NK) cells and their possible role in antitumor surveillance have been extensively reported[8-10]. This papar describes the NK activity of peripheral blood mononuclear

*) 田中義人：急性リンパ性白血病患児のナチュラル・キラー (NK) 活性について

cells from children with ALL at the time of diagnosis and during chemotherapy.

MATERIALS AND METHODS

Children with ALL

This study was carried out on 20 children with ALL aged 1 to 13 years, who were diagnosed at this institute from January 1977 to December 1980. The diagnosis of leukemia was made according to established morphological and cytochemical criteria[11,12]. The membrane markers were examined by E rosette formation[13] and by direct immunofluorescence technique using fluorescein-conjugated polyvalent goat anti-human immunoglobulin (Behringwerke AC, F.G.R.)[14]. Five patients were studied at the time of diagnosis (before starting chemotherapy), and 15 patients remaining in continuous complete remission were studied 1/2-4 years after the initiation of maintenance chemotherapy. The outline of the remission induction and maintenance chemotherapy is shown in Table 1. All patients in remission were examined 1-2 weeks after the chemotherapeutic pulses (on the day of next scheduled chemotherapy). Of these 20 patients, 18 had non-T/non-B acute lymphoblastic leukemia, and 2 had T-cell leukemia.

Informed consent was obtained from patients and/or gurdians.

Cytotoxicity assay

Techniques for the isolation of the effector cells, radiolabeling of the target cells (K 562), and cytotoxicity assay are detailed in previous report[15]. Briefly, the effector cells were isolated from heparinized peripheral blood by centrifugation with Ficoll-diatrizoate sodium gradient. Approximately 2×10^6 target cells were labeled with 100 μCi of ^{51}Cr ($Na_2{}^{51}CrO_4$, 1 mCi/m*l*, specific activity 250-450 μCi/mg Cr, New England Nuclear, Corp, Boston, Mass.) for 50 minutes at 37°C in a humidified CO_2 incubator. The labeled K 562 cells were dispensed into wells of Nunc U-bottomed microtiter plates, each well receiving 0.1 m*l* containing 10^4 cells. Equal volumes of various dilutions of effector cells were added to triplicate wells, yielding ratios of effector to target cells (E/T ratio) of 6.25, 12.5, 25, 50, and 100. The plates were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 3 hours. Incuba-

Table 1. Outline of Tretment protocol

Induction	VCR 2.0 mg/m² iv on days 1, 8, 15, and 22.
	ADR 30 mg/m² iv on day 8.
	PRED 60 mg/m² po daily for 4 weeks.
CNS Prophylaxis	(after complete remission)
	MTX 12 mg/m² & SC 30 mg/m² IT weekly×5.
	MTX 500 mg/m² iv infusion over 24 hours on day 8.
	Cranial irradiation 2,400 rad.
Maintenance	(1) ADR 20 mg/m² & VCR 2.0 mg/m² iv on day 1.
	PRED 100 mg/m²& 6-MP 100 mg/m² po daily for 5 days.
	(2) VCR 2.0 mg/m² iv on day 1.
	MTX 20 mg/m² po on days 1 and 2.
	6-MP 100 mg/m² po daily for 5 days.
	(3) CP 400 mg/m² & VCR 2.0 mg/m² iv on day 1.
	PRED 100 mg/m²& 6-MP 100 mg/m² po daily for 5 days.
	(4) MTX 500 mg/m² iv infusion over 5 hours on day 1.
	Each course is rotated after a rest period of 1-2weeks.

Chemotherapy is terminated after5 years, provided that the patient has continued to remain in complete remission.

VCR=vincristine, ADR=adriamycin, PRED=prednisolone, MTX=methotrexate, SC=Solu-Cortef, 6-MP=6-mercaptopurine, CP=cyclophosphamide, IT=intrathecally.

tion was terminated by centrifuging plates at room temperature, and 0.1 m*l* of supernatant was collected for counting in a well type gamma counter (Shimazu Model RAW-600, Shimazu, Japan).

The NK activity (per cent specific lysis) was calculated from the following formula:

$$\text{per cent specific lysis} = \frac{\text{CPM (experimental)} - \text{CPM (spontaneous)}}{\text{CPM (maximum)} - \text{CPM (spontaneous)}} \times 100$$

where experimental release was determined with effector cells present, and spontaneous release was determined from incubation of target cells in medium only (always less than 20% of the maximum release). The maximum release was determined by adding 0.1 m*l* of 0.5% Triton X−100 to the target.

RESULTS

NK activity at the time of diagnosis

NK activity of the peripheral blood mononuclear cells from 5 children with ALL was evaluated at the time of diagnosis before initiating chemotherapy. As shown in Fig. 1, the NK activity (per cent specific lysis) increased with the E/T ratio. In patients A, B and C, the NK activity was in normal range, and the activity of patients D and E was significantly lower. The effector cell suspensions prepared from patients were always contaminated with various numbers of blast cells. The percentage of this contamination was 3.0%, 57.1%, 65.0%, 80.9%, and 86.3% in patients A, B, C, D, and E, respectively. Considering this contamination, the level of NK activity was inversely proportional to the percentage of blast cells in effector cell populations (Fig. 2). Bone marrow cells aspirated from iliac crests were consisted of almost pure blast cells (more than 94% blasts) in all 5 patients, and these blast cells showed no NK activity against K 562 target cells [per cent specific lysis was 0.2±0.6% (mean±S.D.) (n=5)].

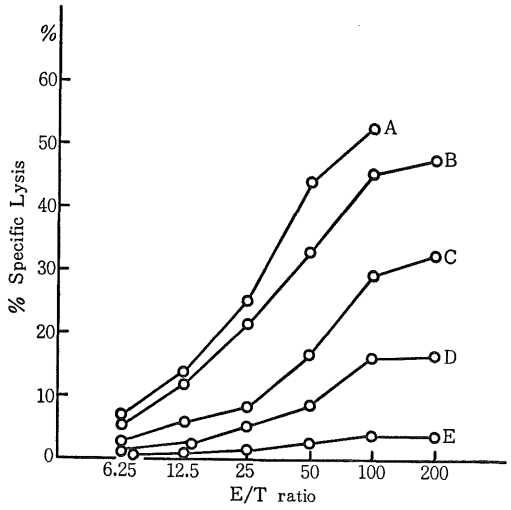

Fig. 1. NK activity at the time of diagnosis of ALL
Before initiating induction chemotherapy, the peripheral blood mononuclear cells from 5 children with ALL were incubated with ^{51}Cr-labeled K 562 cells at E/T ratios of 6.25, 12.5, 25, 50, 100, and 200 for 3 hours.

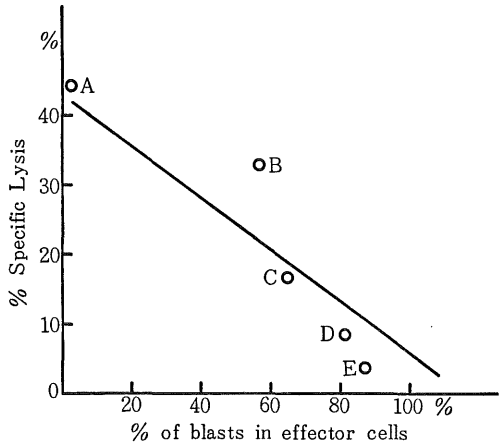

Fig. 2. Relationship of NK activity and percentage of blast cells contaminating effector cell population
The peripheral blood mononuclear cells were reacted with ^{51}Cr-labeled K 562 cells at an E/T ratio of 50 : 1 for 3 hours.

As shown in Fig. 3, after the start of induction chemotherapy with vincristine and prednisolone, NK activity decreased, although the blast cells disappeared from the peripheral blood after 2 weeks of chemotherapy. After complete remission, the NK activity returned to normal level, rather higher than the activity at the time of diagnosis, in patients A, B, C, and E.

NK activity during maintenance chemotherapy

NK activity of 15 children with ALL remaining in continuous complete remission was studied. They had been on intermittent maintenance therapy with multiple chemotherapeutic

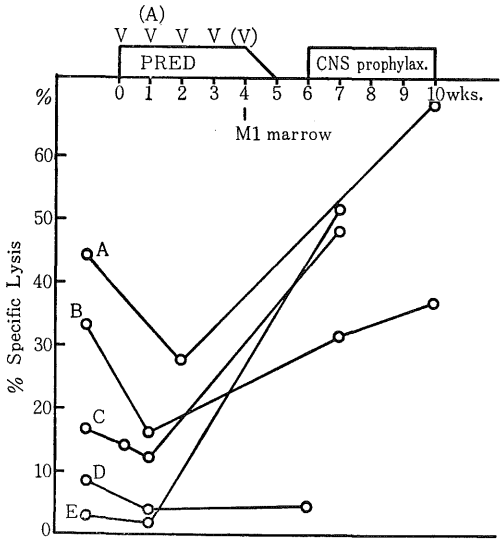

Fig. 3. NK activity during remission induction chemotherapy
The peripheral blood mononuclear cells were incubated with ^{51}Cr-labeled K 562 cells at an E/T ratio of 50 : 1 for 3 hours.

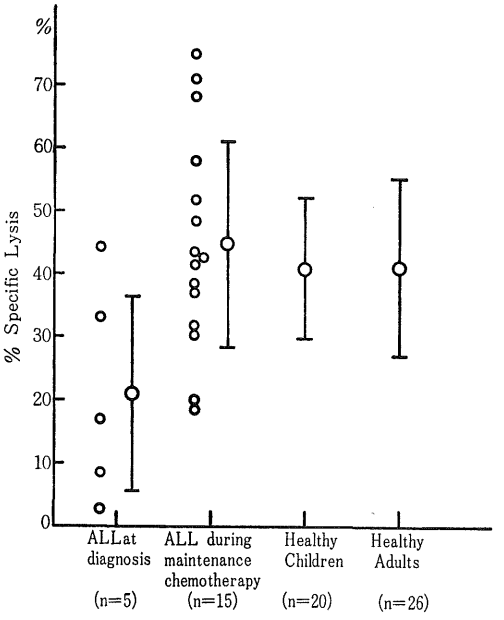

Fig. 4. NK activity of 5 children with ALL at diagnosis, 15 children with ALL during maintenance chemotherapy, 20 healthy children, and 26 healthy adult controls was depicted. The peripheral blood mononuclear cells were incubated with ^{51}Cr-labeled K 562 cells at an E/T ratio of 50 : 1 for 3 hours. The data show the mean % specific lysis ±S. D.

agents as described in Table 1 for 1/2-4 years before the assay was done. Their NK activity (per cent specific lysis) was 44. 9±16. 9% (mean ±S. D.); this was as high as that of 20 healthy children (40. 8±11. 5%) and 26 healthy adults (41. 3±14. 2%) (Fig. 4).

DISCUSSION

The system of immunological surveillance of tumors is thought to be composed of specific cytotoxic T cells, stimulated macrophages, antibody-dependent cellular cytotoxicity (ADCC) with K cells, monocytes, and granulocytes, and natural cell-mediated cytotoxicity of natural killer (NK) cells[16]. The specific cytotoxic T cells with antitumor activity may have minimal role in the very early stages of tumor growth, because they are generated only after a period of tumor growth and consequent sensitization to tumor associated antigen[9]. Macrophages require macrophage-activating factors released from immune T cells, and ADCC requires antitumor antibodies. Therefore, they may come into play more important roles only at later stages of tumor progression. On the other hand, NK cells, which do not require presensitization and exist in normal individuals before bearing tumors, are thought to provide the first order of defence against tumors[9,10].

The NK activity of peripheral blood lymphocytes from healthy nonsensitized individuals have been extensively documented[17-21]. It has been studied in patients with various solid tumors[22-26] and NK cells have been considered to play a role in immunosurveillance against tumors, especially leukemias[27-30]. The NK activity of children with ALL at diagnosis, however, has not been previously reported.

As shown in Figs. 1 and 4, two of the 5 children with ALL showed significantly lower levels of NK activity at the time of diagnosis. The NK activity at this time was inversely proportional to the percentage of blast cells contaminating the effector cell populations; i.e., the larger the number of leukemic blasts in the peripheral circulation the lower the NK activity (Fig. 2). Livnat et al.[31] reported that NK activity of peripheral blood lymphocytes against K 562 cells were subnormal in 35% of leukemia patients before bone marrow transplantation. They speculated that low NK activity in patients

with circulating blasts was probably due to dilution and replacement of normal lymphocytes with blast cells. However, it is also possible that blast cells interfered with NK activity as competitive inhibitors of K 562 cells serving as targets for NK cells, or by exerting a suppressive effect on existing NK cells.

After initiating induction chemotherapy with vincristine and prednisolone, the NK activity of all 5 patients was lower than pre-treatment level, although the number of blast cells in peripheral blood rapidly decreased and they almost disappeared from circulation after 2 weeks of chemotherapy (Fig. 3). Corticosteroids were reported to have suppressive effect on NK activity[32], and after discontinuation of prednisolone, the NK activity of 4 patients increased to level higher than that of the onset (Fig. 3).

As shown in Fig. 4, during maintenance chemotherapy, the NK activity of children with ALL was normal, although multiple chemotherapeutic agents had been given in combination for long periods (1/2-4 years) before NK activity was assayed. Immunosuppressive effects of various chemotherapeutic agents have been well known. Mantovani et al.[33] reported that intraperitoneal injection of azathioprine or cyclophosphamide to mice resulted in marked dose-dependent inhibition of splenic NK activity. The suppressed NK activity recovered rapidly; by 7 days after the treatment, no difference from control values was observed. Oldham et al.[34] reported that children with ALL in remission undergoing maintenance chemotherapy had lower than normal NK activity against F 265 lymphoblastoid cell line. Their patients were treated with continuous daily 6-mercaptopurine and other agents. On the contrary, our patients were treated intermittently with rest periods of 1 to 2 weeks, although the number and dose of chemotherapeutic agents were larger than theirs. For obtaining higher cure rate, the intermittent chemothrapy has been considered to be superior to the daily continuous maintenance therapy[35,36]. Intermittent therapy has the advantage of allowing recovery of the normal hematopoietic cells as well as the recovery of the host's immunologic functions. Although the *in vivo* role of NK cells is not completely understood at present time, it is rationale to maintain NK activity of patients with malignancy at the normal level with the intermittent regimen like ours.

One of the purposes of this study was to decide the prognosis in children with ALL from the level of NK activity. Two patients relapsed during this study, and their NK activity (per cent specific lysis at an E/T ratio of 50 : 1) was 14.3% and 1.0%. The percentage of circulating blast cells at that time was 4.0% and 90.0%, respectively. Unfortunately, their NK activity shortly before the relapse was not examined.

To clarify the correlation between the level of the NK activity and leukemic relapse, more extensive and long-term observation are needed.

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