EXTRACTION OF LEUKEMIA ASSOCIATED ANTIGEN (LAA) AND ACTIVE SPECIFIC IMMUNIZATION WITH LAA IN ACUTE LEUKEMIA

KOHJI EZAKI, M. D.

First Department of Internal Medicine, Nagoya University School of Medicine
(Director: Prof. Itsuro Sobue)

ABSTRACT

Leukemia associated antigen (LAA) was prepared from leukemia cells, using hypotonic lysis and low frequency sonications, followed by diethylaminoethyl (DEAE) cellulose column chromatography. Four protein peaks were eluted by stepwise introduction of increased concentration of NaCl solutions. With running on polyacrylamide gel electrophoresis (PAGE), unique bands which were not present in extracts of remission bone marrow, appeared in eluates of higher mol NaCl solution. These eluates produced positive blastogenic response when incubated with autologous remission lymphocytes. Active specific immunization with pooled allogeneic LAA was performed in 14 adult AML patients in complete remission. Immunization was done weekly for 3 weeks and immunological studies (measurement of in vitro lymphocyte blastogenic responses and delayed hypersensitivity skin reactions) were done weekly for 5 weeks. Twelve out of 14 patients showed increased blastogenic responses to LAA after immunization, and 9 out of 10 studied showed increased blastogenic responses to irradiated autologous leukemia cells. Significant increases in blastogenic responses to both LAA and autologous leukemia cells were noticed on day 22 ($p < 0.05$). The increase of blastogenic responses seems to be higher among the patients whose length of remission was over 12 months at the time of immunization. There was no overall significant difference between blastogenic responses in autologous serum or pooled AB(+) serum. Increased skin test reactivity to LAA after immunization was seen in 7 out of 14 patients. Those patients with an initially weak reaction showed increased reactivity after immunization. There was no correlation between blastogenic responses and skin test reactivity.

INTRODUCTION

Recent studies have shown that tumor associated antigens exist in human acute leukemia as well as in other human malignancies.[1~6] Human leukemia cells have previously been shown to stimulate in vitro lymphocyte blastogenesis.[7~10] It has been demonstrated that after immunization with autologous leukemia cells (autoimmunization), this lymphocyte blastogenesis increased significantly.[11,12]

Since Mathé[13] reported significant prolongation of remission duration in childhood ALL after immunization with irradiated allogeneic leukemia cells plus Bacillus Calmette-Guerin (BCG), several attempts at combined active specific plus active non-specific immunotherapy of acute myelogenous leukemia have been made. The leukemia cells used for active specific immunotherapy were treated with irradiation[14] or neuraminidase[15] or non-treated.[16] Most of these studies have resulted in prolongation of survival duration.

We have produced leukemia associated antigen (LAA) from the cells of patients with acute leukemia, using hypotonic lysis and low frequency sonication, followed by poly-

江崎幸治

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acrylamide gel electrophoresis (PAGE) or diethylaminoethyl (DEAE) cellulose column chromatography.[17] Hollinshead[18,19] has prepared LAA according to this method and obtained positive delayed hypersensitivity reaction when injected intradermally in the autologous setting.

We intend to develop a protocol for active specific immunotherapy of acute leukemia using LAA.[20] Prior to the enforcement of this protocol, we need to identify and evaluate the immunological activity of LAA. In this paper, we present (1) to determine the blastogenic activity of LAA in autologous setting, (2) to demonstrate the boosting of specific tumor immunity after the immunization with pooled allogeneic LAA.

MATERIALS AND METHODS

Collection and storage of leukemia cells

Leukemia cells were collected from the peripheral blood of untreated patients on admission to the hospital. The red blood cells were removed by exposure of the collected cells to five volumes of tris-buffered ammonium chloride. After centrifugation and washing, the leukemia cells were suspended in RPMI-1640 medium with 20% fetal calf serum. This suspension was mixed with DMSO to a final proportion of 10% and frozen at 1°C per minute to −120°C in sterile glass ampules at a leukocyte concentration of 5×10^7/ml and a volume of 1 ml. The ampules were then stored at −180°C in liquid nitrogen.

Preparation of leukemia associated antigen (Fig. 1)

The method of preparation of LAA is essentially the same as previously reported.[17, 20] Leukemia cells were treated with tris-buffered ammonium chloride for removal of erythrocytes and suspended in 0.15 M NaCl. At no time were these cells for immunization with

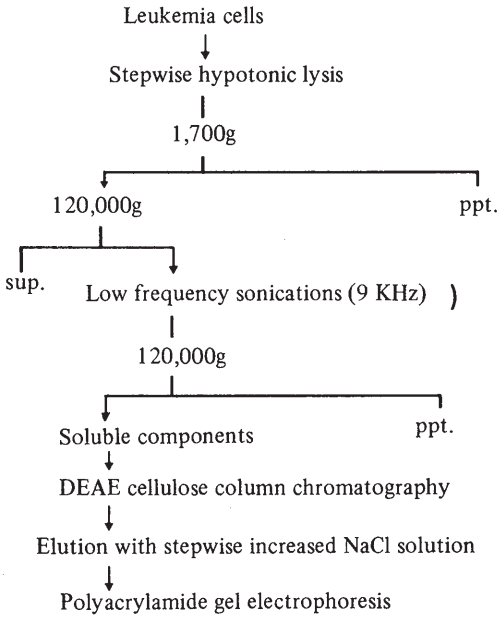

Fig. 1. Preparation of Leukemia Associated Antigen

LAA exposed to fetal calf serum to assure that neither immunization nor response could be attributed to this material. Membrane extracts were prepared by sequential low-ionic strength extraction, with 0.15 M NaCl, 0.07 M NaCl and 0.035 M NaCl. In each extraction, supernatant was collected by centrifugation at 1,700 g for 10 minutes. Supernatants were pooled and then were ultracentrifuged at 120,000 g for 1 hour to obtain the membrane pellet. The membrane pellet was treated with low frequency sonication (Raytheon Sonic Oscillator or Kubota Insonater Model 200M) for 4 minutes, 4 to 6 times to obtain soluble fractions. Thereafter, these soluble fractions were separated on PAGE. The discontinuous gels were stacked gradients of 12, 7, 4.75, and 3.5% acrylamide. In the region close to the anode, there were protein bands noted which were not present in extracts of remission bone marrow (Fig. 2). An alternative to PAGE separation was DEAE cellulose column chromatography. This was used to prepare enough yield of antigen for immunization and immunological studies. Four protein peaks (Peak I, II, III, and IV) were eluted by stepwise introduction of increased concentration of NaCl solutions. The eluated of DEAE run on PAGE showed the unique bands in Peak IV (0.30 M NaCl eluate) and also in Peak III (0.15 M NaCl eluate) in some cases (Fig. 2). Those eluates were concentrated to 500 to 1,000 μg/ml and stored in Revco freezer (−80°C) after sterilization by Millipore filter. LAA was named for the eluates of DEAE showing the unique bands.

Type of immunological studies

a) Lymphocyte blastogenic responses

Peripheral blood lymphocytes were collected on a layer of Ficoll-Conrey by a centrifugation at 400 g for 30 minutes. The lymphocytes were resuspended in RPMI-1640 medium, supplemented with penicillin, streptomycin, glutamin and 10% serum (either patient's own or allogeneic AB(+) serum). Microculture lymphocyte stimulation assay was employed to monitor lymphocyte blastogenic responses. The lymphocyte concentration was adjusted

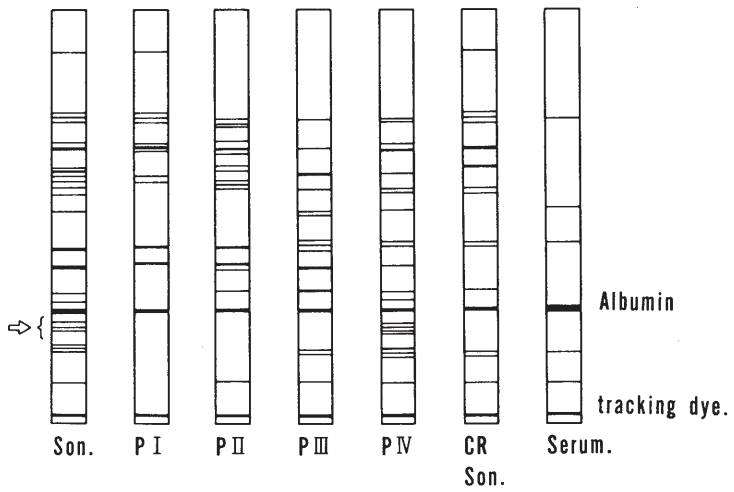

Fig. 2. Polyacrylamide gel electrophoresis. (29 male AML)
Son.: Sonicated prep of leukemia cells
PI, PII, PIII, PIV: DEAE fraction of sonicated prep
CR Son.: Sonicated prep of remission bone marrow cells

to 7.5×10^5/ml for antigen and mitogen treated cultures and 0.2 ml were added to triplicate wells of Falcon microtest II plates. For the cultures of mixed lymphocyte tumor cell reaction (MLTR), the cell concentration was adjusted to 1.5×10^6/ml and 0.1 ml was added to triplicate wells.

LAA was added in the concentrations of 5, 10 and 50 μg/ml of the culture. For MLTR cultures, autologous frozen leukemia cells were thawed, washed and resuspended in RPMI-1640 medium. Leukemia cells were adjusted to 1.5×10^6/ml and after irradiating with 4000 rads, 0.1 ml was added in culture plates to an equivalent number of autologous lymphocytes. Appropriate controls consisting of unstimulated lymphocytes alone and irradiated leukemia cells with irradiated lymphocytes were set up.

Cultures were incubated at 37°C in an atmosphere of 5% of CO_2 in air. Antigen treated cultures were terminated after 5 days and MLTR cultures were terminated after 7 days of incubation. During the final 8 hours of incubation, 1 μCi/well of tritiated thymidine was added to the cultures. Cells from each microculture were harvested on MASH (multiple automated sample harvester) onto fiberglass scintillation paper. Circular discs containing cells were removed and transferred to glass vials containing scintillation fluid. These were counted in a scintillation counter. Lymphocyte blastogenic responses were taken as the net CPM per 1.5×10^5 lymphocytes after the results of the appropriate control cultures had been subtracted. The stimulation index (SI) was taken as the CPM in a stimulated culture divided by the CPM in the appropriate unstimulated control.

b) Delayed hypersensitivity skin reactions

Delayed hypersensitivity was evaluated by injecting 0.1 ml intradermally at 2 different concentrations of LAA (10 and 100 μg/ml) with resulting skin test doses of 1 and 10 μg, respectively.

We have used MLTR to detect leukemia cell surface antigen,[10] and so lymphocyte blastogenic responses were thought to be useful and performed in autologous setting to evaluate the antigenic acitvity of each eluate of DEAE. Leukemia cell extracts were collected from leukemia cells of the patients before treatment. After these patients entered into complete remission, the lymphocytes were collected and stimulated with autologous eluates of DEAE. The study was done at least 1 week after the last chemotherapy to avoid the immunosuppressive effects of chemotherapeutic agents. These lymphocyte blastogenic responses were done in 5 AML patients and 4 ALL patients whose remission duration was 2 to 4 months.

Schedule of immunization and immunological studies

Fourteen patients with AML were immunized with pooled allogeneic LAA. LAA used for immunization were prepared from pooled leukemia cells of 8 AML patients. All patients were in complete remission with a duration of remission between 3 months and 18 months, and a median remission duration of 7 months. Immunization was started 2 to 3 weeks after the last chemotherapy to avoid the rebound period,[21] and immunosuppressive effects of chemotherapeutic agents.[22]

On the day of immunization, LAA which had been stored in Revco freezer, were thawed rapidly at 37°C, and the dose was adjusted to either 100, 200, or 400 μg in 0.2 ml. The dose was increased as it was demonstrated that the lower doses were safe and not associated with toxicity. LAA was mixed with 50 μg/0.05 ml of oil-attached BCG-cell

wall skeleton (BCG-CWS) as an adjuvant and injected intradermally at 2 separate sites in one extremity on days 1, 8, and 15 of the study. The sites of immunization were rotated in both upper and lower extremities. The patients did not take any other treatment during these 3 immunizations and subsequent 2 weeks follow-up. Chemoimmunotherapy or chemotherapy maintenance was resumed 2 weeks after last active immunization and after the end of the immunological studies.

Immunological studies included; 1) lymphocyte blastogenic responses to LAA and irradiated autologous leukemia cells (MLTR); 2) lymphocyte blastogenic responses to nonspecific mitogens: phytohemagglutinin (PHA-M, Difco), pokeweed mitogen (PWM, Gibco), concanavalin-A (Con-A, Pharmacia), streptolysin-O (SLO, Difco), and preservative-free PPD. Lymphocyte cultures were done using patient's own serum and allogeneic AB(+) male serum; 3) skin tests for delayed hypersensitivity to LAA, as well as nonspecific mitogens: dermatophytin, varidase, candida, PPD, and keyhole limpet hemocyanin (KLH).

Lymphocyte blastogenic responses were measured on the first day of immunization and then weekly for 4 weeks, that is, days 1, 8, 15, 22, and 29 of the study. Skin tests were done on the first day, and then every other week for 4 weeks, that is, days 1, 15, and 29 of study.

RESULTS

Lymphocyte blastogenic responses in autologous setting

Lymphocyte blastogenic responses to the eluates of DEAE in autologous setting showed in Table 1. Four out of 5 AML patients, and 4 out of 4 ALL patients showed higher responses to either Peak IV or Peak III and Peak IV, than to other peaks. Mean CPM of all patients when incubated with Peak IV and Peak III is 2938 and 2618, respectively. Compared to unstimulated control, blastogenic response only to Peak IV is significantly

Table 1. Lymphocyte blastogenic responses to each DEAE fraction in autologous setting

Pt.	Dx.	Blastogenic responses CPM (SE)				
		None (Control)	PI	PII	PIII	PIV
M.M.	AML	504 (88)*	903 (120)	774 (192)	737 (192)	1252 (161)
H.W.	AML	2794 (420)	2134 (95)	3774 (946)	5400 (1453)	4768 (497)
K.G.	AML	1136 (321)	1919 (690)	1542 (388)	1774 (172)	2678 (280)
Y.I.	AML	1680 (400)	3933 (2292)	1806 (1117)	5832 (1726)	6287 (992)
M.M.	AML	1183 (233)	797 (124)	961 (213)	1129 (193)	1253 (337)
K.H.	ALL	1410 (188)	1307 (627)	1432 (623)	1630 (442)	2105 (752)
K.B.	ALL	702 (107)	1525 (495)	1208 (216)	2014 (747)	1719 (409)
T.H.	ALL	1950 (248)	2951 (50)	3339 (423)	3184 (427)	4052 (290)
Y.H.	ALL	362 (39)	512 (119)	1615 (181)	1869 (317)	2328 (432)
Mean CPM		1302	1775	1827	2618	2938
P-value			<0.25	<0.25	<0.10	<0.05

* SE = Standard error.

higher ($p < 0.05$). Blastogenic responses to unfractionated sonicated materials are lower than the blastogenic responses to Peak IV in many cases.

Immunization with allogeneic LAA

Among the 14 AML patients immunized with LAA, 6 patients received 100 μg, 5 patients 200 μg, and 3 patients 400 μg, respectively. All patients tolerated the immunization well. Most patients had moderate local reactions at the injection sites consisting of approximately 1 cm of erythema, induration, and papule formation lasting for 1 week. Only 3 patients developed mild temperature elevation. These 3 also developed more severe local reactions at injection sites including drainage which lasted for 2 to 3 weeks.

Table 2 summarizes the pre- and post-immunization lymphocyte blastogenic responses to LAA in both autologous and allogeneic serum. The highest counts among 3 different concentrations of LAA used for lymphocyte stimulation are shown, and the highest values among 4 in vitro studies after immunization and the dates on which the highest values were observed are given. After immunization with LAA, 12 out of 14 patients showed a significant increased blastogenic response to LAA at least twice the pre-immunization value. The other 2 patients did not show a significant increase. In the majority of cases, the highest responses appeared on days 15, 22, and 29 of the study.

Figure 3 summarizes the net CPM and SI to LAA of all patients who were immunized with LAA. Statistical analysis by Wilcoxon paired t test showed significant differences between day 1 and day 22 ($p < 0.01$). Because lymphocyte blastogenic responses in allogeneic serum were similar, only the result using autologous serum are shown in the figure.

Table 2. Pre- and Post-Immunization Blastogenic Responses to LAA

Patient[a]	Blastogenic Responses to LAA (Net CPM)					
	Auto Serum[b]			Allo Serum[b]		
	Pre	Post[b]	(Day)[d]	Pre	Post[c]	(Day)[d]
1.	60	982	(22)	89	895	(22)
2.	312	391	(22)	281	559	(29)
3.	160	386	(22)	339	700	(22)
4.	692	1407	(22)	899	2277	(22)
5.	498	1846	(29)	539	1261	(15)
6.	1601	4531	(22)	3064	13228	(22)
7.	1320	8127	(29)	520	4929	(29)
8.	766	2145	(8)	1370	2759	(8)
9.	1458	2018	(22)	1729	6636	(29)
10.	1006	10840	(29)	608	6932	(29)
11.	−697	207	(15)	−357	323	(22)
12.	3228	7343	(22)	3318	2005	(8)
13.	4899	13436	(22)	4677	18501	(22)
14.	7530	6860	(22)	7284	6936	(22)

a) Doses of LAA for immunization are 100 μg (Pt. 1–6), 200 μg (Pt. 7–11), and 400 μg (Pt. 12–14).

b) Serum used for in vitro lymphocyte cultures.

c) Highest net CPM among 4 studies after immunization.

d) Day with the highest net CPM.

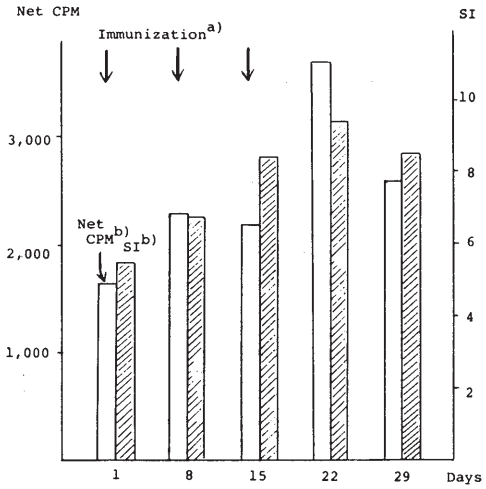

Fig. 3. Changes in Blastogenic Responses to LAA

a) Immunization was given on days 1, 8, and 15.

b) Net CPM and SI: Median value of lymphocyte blastogenic responses using autologous serum in 14 immunized patients.

Significant differrence between day 1 and day 22 ($p<0.01$) by Wilcoxon paired t test.

Table 3. Pre- and Post-Immunization Blastogenic Responses to Autologous Leukemia Cells

Patient[a]	Blastogenic Responses to Cells (Net CPM)					
	Auto Serum[b]			Allo Serum[b]		
	Pre	Post[c]	(Day)[d]	Pre	Post[c]	(Day)[d]
1.	5000	10502	(8)	4399	10467	(29)
3.	676	503	(8)	285	897	(15)
4.	420	1635	(29)	1249	1680	(8)
5.	702	1446	(29)	799	1677	(29)
6.	1256	10025	(22)	698	10120	(22)
8.	584	13791	(22)	684	17881	(22)
9.	73	705	(22)	667	3626	(15)
12.	3055	12237	(29)	6729	14121	(22)
13.	126	6066	(8)	152	1314	(22)
14.	1064	1015	(29)	847	820	(22)

a) Doses of LAA for immunization are 100μg (Pt. 1, 3, 4, 5, 6), 200μg (Pt. 8, 9), and 400μg (Pt. 12, 13, 14).
b) Serum used for in vitro lymphocyte cultures.
c) Highest net CPM among 4 studies after immunization.
d) Day with the highest net CPM.

The pre- and post-immunization lymphocyte blastogenic responses to irradiated autologous leukemia cells after immunization are shown in Table 3. Autologous leukemia cells were available in only 10 out of 14 patients. Nine out of 10 patients showed a significant increased blastogenic responses to autologous leukemia cells after immunization.

Figure 4 summarizes the blastogenic responses to autologous leukemia cells by net CPM and SI. Significant increases were noted on day 8 ($p < 0.05$) and day 22 ($p < 0.05$).

The lymphocyte blastogenic responses to non-specific mitogens after active specific immunization are shown in Figure 5. There were no significant changes in the responses to PHA, PWM, Con-A, or SLO. In vitro lymphocyte blastogenesis to PPD was also studied because of the possibility of crossreactive antigen between AML cells and BCG, but there was no increase in stimulation noted.

There was some correlation between the length of remission at the time of the study and increase in lymphocyte blastogenic responses. All 4 patients whose length of remission (patient 1, 6, 7, 13) was over 12 months developed 5-fold or greater blastogenic responses to LAA or autologous leukemia cells after immunization. In contrast, only 2 out of 6 (patient 8, 9) and 1 out of 4 (patient 10) whose length of remission were 3 to 6 months and 7 to 11 months, respectively, developed 5-fold or greater blastogenic responses. There seems to be no correlation between the initial net CPM and the increase in blastogenic responses following active immunization. There was no statistical difference (by chi square analysis) of the increase of blastogenic responses between initial net CPM of $\geqq$ 1,000 and $<$ 1,000.

Of 14 patients who received LAA, 7 patients had 100%or more increase in skin test reactivity to LAA. When we group the patients based on their initial pre-immunization skin test reactivity to LAA, 7 had more than 10 mm, while 7 had less than 10 mm of induration. None of the 7 patients with vigorous initial reactivity showed an increase after immunization. In contrast, all 7 patients whose skin test reactivity was less than 10 mm showed a significant increase. Skin test reactivities to 1 or more nonspecific recall antigens were noticed to have increased following active specific immunization. The antigens which caused increased skin test reactivity were not always the same in each patient. Of 7 patients who showed increased skin test reactivity to LAA, 6 patients also showed increased reacti-

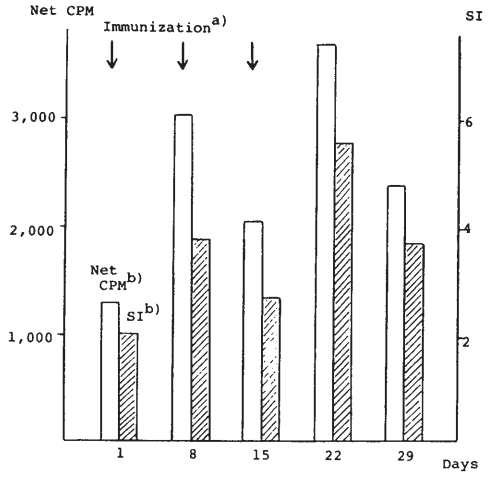

Fig. 4. Changes in Blastogenic Responses to Autologous Leukemia Cells

a) Immunization was given on days 1, 8, and 15.

b) Net CPM and SI; Median value of lymphocyte blastogenic responses using autologous serum in 10 immunized patients.

Significant difference between day 1 and day 8 ($p<0.05$) and day 1 and day 22 ($p<0.05$).

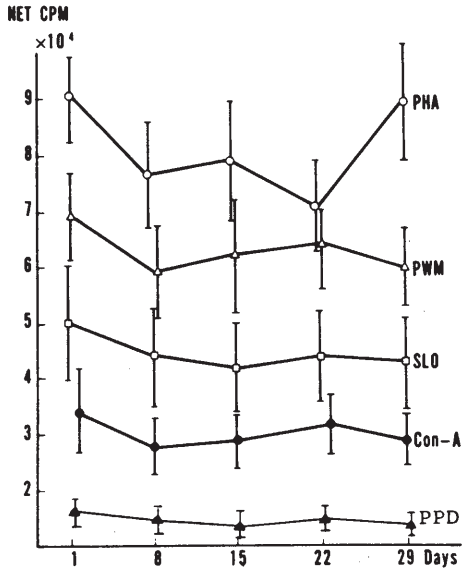

Fig. 5. Changes in Blastogenic Responses to Non-specific Mitogens after Immunization with LAA

vity to 1 or more recall antigens (Table 4). Thus, there was no selective increase of skin test reactivity to LAA after active immunization. There seems to be no correlation as determined by statistical analysis (chi square) between lymphocyte blastogenic response and skin test reactivity to LAA. Of 12 patients who had increased lymphocyte blastogenic responses to LAA, only 5 showed increased skin test reactivity to LAA. Of 2 patients who did not have increased blastogenic responses to LAA, all showed increased skin test reactivity to LAA.

DISCUSSION

The presence of tumor antigens and tumor immunity have been demonstrated in leukemia by a variety of techniques for cell-mediated and/or humoral immunity.[23] Several attempts have been made to solubilize human leukemia associated antigens, by using Papain digestion,[24] KCl extraction,[8] low frequency sonication.[18] Hollinshead[19] had prepared purified acute leukemia associated antigens, by using hypotonic lysis, low frequency sonication, and column chromatography, followed by discontinuous PAGE. This permitted the separation of some of the normal tissue antigens from the tumor associated antigens. These antigens appeared to be of low molecular weight and in the rapidly migrating region. The proteins eluted from PAGE region III produced positive skin test reactions. Comparable preparations of remission cells gave negative skin reactions. In human study, we can not use the method of immunoprophylaxis like in animal study, and it is obvious that there is no definite way to detect the antigenic activity of tumor cell extract. Our present study using lymphocyte blastogenic responses showed higher response to Peak IV and also Peak III in some cases than the response to other peaks, which suggested that the antigenic activity will be in these peaks. These peaks contain unique bands on PAGE pattern, and our data of lymphocyte blastogenic responses may correlate with the results of delayed hypersensitivity reaction by Hollinshead.[19] It is possible that the unique bands appeared on PAGE caused positive lymphocyte blastogenic response and delayed hypersensitivity reaction as well.

The most effective form of immunotherapy in L1210 leukemia in mice is immunization with a combination of BCG and irradiated tumor cells.[13] Mathé[13] used this type of treatment in the maintenance of remission in childhood ALL. Powles[14] used BCG and irradiated allogeneic leukemia cells for maintenance treatment of AML with prolongation of remission duration and survival. In addition to benefit of BCG plus irradiated allogeneic leukemia cells, Hamilton Failey[16] observed that patients who received BCG plus nonirradiated

Tab. 4 Correlation between Skin Test Reactivity to LAA and Skin Test Reactivity to Recall Antigens

Changes of Skin Test Reactivity to LAA	No. of Patients	No. of Increased Skin Test Reactivity to Recall Antigens[a]					
		0	1	2	3	4	5
Increased	7	1	3	1	2	0	0
No change or Decreased	7	2	4	1	0	0	0

a) Dermatophytin, Varidase, Candida, PPD, and KLH.

leukemia cells who had achieved only partial remission, had better survival than the patients receiving chemotherapy alone who achieved complete remission. Holland[15] administered neuraminidase-treated allogeneic leukemia cells and noticed striking prolongation of remission and survival.

Thus, allogeneic leukemia cells have been used for active specific immunotherapy of acute leukemia and the results have been usually positive, at least in AML. However, few if any investigators have observed changes in tumor associated autologous immunity following active specific immunization with either allogeneic leukemia cells or leukemia cell extracts. Gutterman[11] observed that 10 out of 15 patients had increased blastogenic responses to autologous cells after immunization with autologous living leukemia cells. Powles[12] reported the same findings by immunizing with irradiated autologous leukemia cells. Both reports noticed increased blastogenic responses to nonspecific mitogens as well.

In our present study, after immunization with LAA, we noticed an increase of tumor associated cell-mediated immunity without an increase of non-tumor specific immunity as measured by lymphocyte blastogenic responses. It appears that immunization with LAA had specifically boosted the tumor associated cell-mediated immune responses of these patients. It is not yet clear what the relationship between increased blastogenic responses to LAA or autologous leukemia cells and clinical course will be. It is possible that increased blastogenic responses may be associated with a good clinical course, since, as previously observed, high blastogenic responses to autologous leukemia cells correlate with a good prognosis.[8] In contrast, no selectivity of response to LAA with regard to delayed hypersensitivity was noted. Recall antigen responses increased as much as LAA responses.

The most important feature of this study was the increased blastogenic responses to autologous leukemia cells when the patients were immunized with LAA from pooled allogeneic leukemia cells. This suggests that a shared common antigen in the leukemias, particularly in AML, is present.

The study design used here has several defects. First, there was no unimmunized control group, but rather each patient served as his own control. Second, since the patients were recently on chemotherapy, the rebound recovery might explain the blastogenesis results.[21] Third, since the antigen was mixed with an adjuvant, the adjuvant alone could have been responsible for the results and an adjuvant control should have been considered. Because of the limited number of patients available, untreated or adjuvant control could not be done. The stability of the blastogenic responses to the other stimulants suggests that the patients were suitable auto controls and that post-chemotherapy rebound did not explain the results.

In this study, there is no correlation between lymphocyte blastogenic response and skin test reactivity to LAA. This might be expected because these assays probably deal with different phases of immunological response. Herberman[25] observed that there was no correlation among three assays for the evaluation of cell-mediated immunity, namely, MLTR, leukocyte cytotoxicity, and skin test reactivity to tumor extract. Also, he observed that skin test reactivity correlates with clinical state of disease. At this stage of our study, we are not in a position to cerrelate blastogenic response and skin test reactivity to the clinical course due to the limited time of follow-up and the very few patients on the study who have relapsed or died.

In the study we observed that the patients who have been in remission for more than 12 months seem to have higher blastogenic responses after active immunization than those who

have been in remission for less than 12 months. There are several explanations for this. First, it is possible that the patients who have recently entered remission may still be in an immunosuppressed state due to chemotherapy. While all patients in this study were immunocompetent as judged by their in vitro blastogenic responses and in vivo skin test reactivity, their cell-mediated immunity to LAA may have been depressed. Second, during early stage of complete remission, tumor associated immune reaction may still be suppressed selectively, perhaps because of antigen overload, and response to active immunization as secondary response may be low. On the other hand, the patients who have long remission duration may now be able to respond to active immunization as secondary response. Finally, it has been observed that the tumor associated immunity declines with increasing remission duration in animal tumor systems using MLTR.[26] Active specific immunization may be useful during this state of declining tumor associated immunity. The increased skin test reactivity to LAA was seen in the patients with initially small skin test reactivity.

The significant increase of tumor associated immunity by lymphocyte blastogenic responses was seen on day 22, on week after the last immunization, which declined on day 29. Since we did not follow the immunological studies thereafter, because maintenance chemotherapy was resumed, it was not possible to know the outcome of the immune responses. However, it is likely that the tumor associated immunity does not last long after immunization, as suggested by the waning immune response on day 29. Thus, it may be reasonable to give active immunization at weekly intervals in order to maintain heightened levels of tumor associated immunity.

We used BCG-CWS as adjuvant for active immunization and we did not observe the significant increase of lymphocyte blastogenic responses to PPD nor delayed hypersensitivity reaction to PPD after active immunization. It may be possible that the dose of this adjuvant used is not enough to augment the in vitro or in vivo responses to PPD. At present, it is also not clear what is the optimal dose of LAA for effective immunization. In addition, the proportion of antigen and adjuvant necessary for good response is unknown. It is imperative to evaluate the effective dose responses in clinical trial with active immunotherapy.

There are several reports in which autologous serum inhibited the lymphocyte blastogenic response due to inhibitory serum factors.[27,28] We could not find significant differences in blastogenic responses using autologous serum and allogeneic normal AB(+) serum system. Probably, these factors are absent or decreased in serum from remission patients. It would be interesting to study the effect of patients' serum before treatment or during relapse.

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Some data in this article have been published in Cancer vol. 41, 1978. I have cited those data for better understanding of my study after getting the permission of citation from Dr. Evan M. Hersh who is a co-author of my previous article and a board of director of American Society of Clinical Oncology.

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