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Antigen Solubilized from Human Leukemia: Lymphocyte Stimulation

Abstract. *Soluble antigen was extracted with hypertonic (3 molar) potassium chloride from the malignant cells of seven patients with acute leukemia. The antigen and leukemia cells were used to stimulate autologous patients' and allogeneic normal donors' lymphocytes in mixed lymphocyte cultures. The lymphocytes of six patients showed significant blastogenic responses to autologous antigen. In contrast, the lymphocytes of only one of seven normal donors responded to the soluble antigens. Both patients' and normal subjects' lymphocytes responded to the intact leukemia cells. The use of these antigens should facilitate the study of specific tumor immunity in human leukemia.*

The existence of tumor-associated antigens on human leukemic cells has been suggested by the reports of positive lymphocyte responses to autologous leukemia cells in mixed lymphocyte cultures (*1*, *2*). A point of debate is whether a positive blastogenic response to autologous leukemia cells represents a primary response in vitro or whether it actually represents evidence of preexisting immunity (*1*). As a result of the reported successful extraction of tumor-associated antigens from guinea pig hepatoma cells with hypertonic KCl (*3*), we have applied similar techniques to the extraction of soluble antigens from human leukemia cells. These soluble preparations have been assayed in lymphocyte cultures with both autologous leukemic patients' and allogeneic normal donors' lymphocytes. In addition to demonstrating the feasibility of salt extraction of antigens from human leukemic cells, our data suggest that the in vitro lymphocyte responsiveness represents specific tumor immunity.

Blast cells were collected from the peripheral bloods of seven leukemia patients on admission to the hospital. The cells were collected with the IBM or the Aminco blood cell separators (*4*). The red blood cells were removed by exposure of the collected cells to five volumes of tris-buffered ammonium chloride (*5*). The leukemia cells were suspended in media with 10 percent fetal bovine serum and 10 percent dimethyl sulfoxide and frozen and stored in liquid nitrogen.

For extraction of tumor antigen, the method described by Meltzer and coworkers (*3*) was used with some modifications. Depending on the availability of cells, 3×10^8 to 3×10^{10} leukemia cells were used. Ten milliliters of 3*M* KCl in potassium phosphate–buffered saline at *p*H 7.2 were added to every 3×10^8 cells in screw-cap glass tubes 25 by 200 mm. This mixture was equilibrated at 4°C for 16 hours. The contents were then centrifuged at 100,000*g* for 60 minutes at 4°C. The supernatant was dialyzed against 20 volumes of potassium phosphate–buffered saline for 24 hours, with a change of solution every 8 hours. The dialyzed extract was centrifuged at 40,000*g* for 15 minutes at 4°C. The supernatant was concentrated by ultrafiltration at 4°C, and the concentrate was mixed with an equal volume of 3.8*M* ammonium sulfate for 1 hour at 4°C to precipitate the protein. This mixture was centrifuged at 40,000*g* for 15 minutes at 4°C. The precipitate was dissolved in 5 ml of phosphate-buffered saline, *p*H 7.2, and dialyzed against this buffer for 4 hours. The protein concentration was estimated by measurement of optical density at a wavelength of 280 nm. The final preparation was sterilized by filtration through an 0.45-μm Millipore filter and stored at 4°C.

For the lymphocyte culture studies, peripheral blood lymphocytes were

Table 1. Blastogenic response to autologous leukemia cells (LC) and soluble leukemia cell antigen.

Cell and antigen number	Type of leukemia	Incorporation of ^{3}H* (10^3 count/min)		Stimulation index*		Effect of therapy†
		LC	Antigen	LC	Antigen	
1	AML	1.7	2.4	3.1	5.4	R
2	AML	5.9	21.8	10.8	39.6	R
3	AML	9.2	1.0	30.5	4.4	R
4	AML	19.5	1.6	38.0	6.4	R
5	AML	0	0.62	0	1.1	R
6	ALL	0.72	0.56	3.6	2.8	F
7	CML-BC	0	0.64	0	3.1	F

* Maximum response in autologous or allogeneic serum. † R, remission; F, failure.

collected, washed, and cultured (*6*). Cultures contained 1×10^6 washed lymphocytes, 1 ml of serum (either autologous—drawn on the day of study—or allogeneic), 2 ml of Eagle minimal essential medium, and leukemia cells or soluble antigen as stimulants. The stimulator leukemia cells were added in doses from 10^4 to 10^6 in half-log increments; both unirradiated and irradiated (4000 rads) cells were used. Soluble tumor antigen was added in amounts ranging from 10 to 1000 μg per milliliter of culture. Cultures were incubated at 37°C in an atmosphere of 5 percent CO_2 and air for 5 days (tumor antigen) or 7 days (tumor cells). Harvesting was accomplished as described (*6*). During the last 3 hours of incubation 2 μc of [^{3}H]thymidine (specific activity, 1.9 c/mmole; Schwarz BioResearch) was added. The blastogenic responses were determined by the incorporation of the isotope into the acid-insoluble fraction as measured by liquid scintillation counting and expressed as the number of counts per minute per 10^6 lymphocytes. The stimulation index was defined as the number of counts per minute of a stimulated culture divided by the counts per minute of the appropriate unstimulated lymphocyte culture. A 100 percent increase or 50 percent decrease in thymidine incorporation, compared to the appropriate controls, was significant stimulation or inhibition at the 95 percent level of confidence (*7*). A 200 percent increase or 75 percent decrease was significant at the 99 percent level of confidence.

Table 1 summarizes the patients' lymphocyte blastogenic responses to autologous leukemia cells and soluble antigen. Five of the patients had acute myelogenous leukemia (AML), one had acute lymphoblastic leukemia (ALL), and one had blast crisis (BC) of chronic myelogenous leukemia (CML). The patients were studied as soon as their peripheral blood was cleared of leukemic cells by regimens of intermittent chemotherapy, just prior to a course of chemotherapy and at least 5 days after the termination of the previous course. Four of the five patients with AML and the patient with ALL had significant blastogenic responses to autologous leukemia cells. All five of these patients as well as the patient with blast crisis had significant blastogenic responses to soluble antigen. The response to soluble antigen was greater than the response to cells in patients 2 and 7. In contrast, the response to intact leukemia cells was greater in patients 3 and 4. Four of the five patients achieving remission responded to antigen with a stimulation index greater than 4. In contrast, the two patients who failed to achieve a bone marrow remission had stimulation indices less than 4.

Table 2. Blastogenic response of normal human lymphocytes to allogeneic leukemia cells (LC) and soluble leukemia cell antigen.

Cell and antigen number	Incorporation of ^{3}H (10^3 count/min)		Stimulation index	
	LC	Antigen	LC	Antigen
1	26.0	0.53	75.4	2.7
2	30.0	0.08	60.0	1.2
3	18.0	0	12.5	0.1
4	65.0	0	65.0	0.7
5	25.0	0	52.0	0.7
6	0.17	0.18	1.6	1.6
7	34.0	0.10	66.0	1.2

The blastogenic response of normal allogeneic donor lymphocytes to leukemia cells and soluble antigen is shown in Table 2. Six of the seven leukemic cell preparations elicited a vigorous blastogenic response in vitro. In contrast, only one of the soluble antigen preparations (No. 1) induced a significant response, and this was quite weak.

Although dose-response relationships were noted in these studies, good stimulation was noted with doses as low as 10 to 100 μg/ml. Because of the limited availability of the antigen, doses higher than 1000 μg/ml of culture were not used. In at least two patients, the maximum stimulating dose of antigen may not have been used since the dose-response curve was still rising at 1000 μg/ml.

These data indicate that antigen extracted from human leukemia cells by 3*M* KCl can be used to stimulate autologous lymphocytes in lymphocyte cultures. The results correspond quite well with the lymphocyte response to autologous leukemia cells.

Except for one report to the contrary (*8*), most workers have reported that soluble histocompatible antigens can stimulate lymphocyte blastogenesis only after prior sensitization (*9*). Only one of the normal donors in our study responded to the allogeneic soluble antigen preparations, and even that response was very weak, while six of the seven leukemia patients responded to their autologous antigen. Thus, the data support the idea that this lymphocyte stimulation indicates prior sensitization to the antigen and represents specific tumor immunity. A similar observation has been made in a patient with carcinoma of the colon (*10*). Whether prior sensitization is required for in vitro blastogenic response to cell-associated leukemia antigen is still open to question (*11*).

Thus, our study has demonstrated the usefulness of soluble antigen in the study of tumor immunity in patients with leukemia. The use of soluble antigen should identify subjects previously sensitized with leukemia-associated antigens and may be helpful in the search for common antigens on leukemia cells. In addition, such antigen preparations should be useful in other assays of cell-mediated immunity such as migration inhibition and delayed hypersensitivity.

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