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IMMUNOGLOBULIN ON TUMOR CELLS AND TUMOR-INDUCED LYMPHOCYTE BLASTOGENESIS IN HUMAN ACUTE LEUKEMIA

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Abstract Combined studies of direct membrane immunofluorescence with anti-immunoglobulin serum and of lymphocyte blastogenic responses to autologous leukemia cells were carried out in 34 adult patients with acute leukemia. Twenty-four of 34 (71 per cent) has positive blastogenic responses to their own leukemia cells. Eight of 19 patients with acute myelogenous leukemia and one of five with acute lymphoblastic leukemia had complete or partial abrogation of this positive blastogenesis when the lymphocytes were cultured in autologous rather than allogeneic serum. Direct membrane immunofluorescence with anti-immunoglobulin serum showed bound IgG on the cells of seven of eight patients with acute myelogenous leukemia and serum inhibition, and one without the serum inhibitory effect. Membrane immunofluorescence was negative in two patients with positive blastogenesis and serum inhibition, 14 of 15 with positive blastogenesis and no serum inhibition and, finally, all 10 patients with negative blastogenic responses to leukemia cells. A good prognosis was correlated with a positive blastogenic response, its inhibition by autologous serum, and IgG bound to the cell surface.

DURING the past several years there has been increasing evidence that tumor-associated antigens are present in a variety of human neoplasms,[1,2] including acute leukemia. In studying the immunologic response to human leukemia, Yoshida and Imai found that the serum of 71 per cent of patients with acute leukemia gave a positive immune adherence reaction with autologous leukemia cells.[3] In contrast, using similar methods, Doré et al. observed that only 22 per cent of 140 patients with acute leukemia had evidence of antibody directed against autologous leukemia cells.[4] Cell-mediated immunity was observed by Oren and Herberman, who reported that the majority of patients with acute leukemia had positive skin tests to membrane preparations of their own leukemia cells.[5] Cellular reactivity to leukemia has also been investigated in vitro by the mixed-leukocyte-culture technic and by lymphocyte cytotoxicity. Both in vitro lymphocyte blastogenic responsiveness and lymphocyte cytotoxic reactions to autologous leukemia cells have been demonstrated in patients with acute leukemia.[6-8]

Of possible relevance to the apparent failure of this tumor immunity to control clinical cancer was the demonstration that serum factors in patients with solid tumors may interfere with in vitro cell-mediated immunity.[9] The Hellströms and their co-workers have described a factor (presumably antitumor antibody) in the serum of patients with solid tumors that may block lymphocyte-mediated cytotoxicity to tumor cells.[10]

Because there have been few studies of the role of serum factors in tumor immunity in patients with acute leukemia, the current study was designed to determine the presence and possible inter-relation of humoral and cell-associated tumor immunity in acute leukemia. Evidence for both humoral and cell-mediated tumor immunity was found. The presence of a vigorous lymphocyte blastogenic response to leukemia cells, its inhibition by autologous serum and the presence of immunoglobulin bound to the leukemia cells were observed to correlate with a good prognosis.

MATERIALS AND METHODS

Thirty-four patients with acute leukemia had concurrent studies of membrane immunofluorescence with anti-immunoglobulin serum and blastogenic response to autologous leukemia cells. The blastogenic response was studied in both autologous and allogeneic normal serum.

Leukemic blast cells were collected from the peripheral blood of untreated patients on admission to the hospital. The cells were collected with the IBM or the Aminco blood cell separators as previously used to obtain lymphocytes.[11] The red blood cells were removed by exposure of the collected cells to 5 vol of Tris-buffered ammonium chloride.[12] After centrifugation and washing, the leukemia cells were resuspended in RPMI 1640 with 20 per cent fetal calf

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serum. This suspension was mixed with a 10 per cent volume of dimethyl sulfoxide and frozen at 1°C per minute to −120°C in sterile glass ampoules at a leukocyte concentration of 5×10^7 per milliliter and in a volume of 1 ml. The ampoules were thawed rapidly at 37°C, washed 3 times in Spinner Modified Eagle's minimal essential medium (Hyland Laboratories, Los Angeles, California) and resuspended in the same medium at room temperature. The cells were counted in a hemocytometer chamber, and viability was determined by trypan blue dye exclusion.[14]

For the lymphocyte culture and other lymphocyte studies, peripheral blood lymphocytes were collected from treated patients, washed and cultured as previously described.[11] Cultures contained 1×10^6 washed lymphocytes, 1 ml of serum (either autologous or allogeneic), 2 ml of the modified Eagle's medium and stimulants consisting of leukemia cells, phytohemagglutinin (Difco Laboratories, Detroit, Michigan), or allogeneic normal leukocytes. The allogeneic serum was pooled normal human serum. Autologous serum used for a particular experiment was obtained at the same time as the lymphocytes. The stimulator leukemia cells were added in doses varying from 10^4 to 10^6 in half-log increments. Both unirradiated and irradiated cells (4000 or 12,000 R) were used in every experiment. Appropriate controls consisting of unstimulated lymphocytes, unirradiated leukemia cells cultured alone, irradiated leukemia cells cultured alone and irradiated leukemia cells cultured with irradiated lymphocytes were set up. Cultures were incubated at 37°C in an atmosphere of 5 per cent carbon dioxide in air for 3 to 5 days (phytohemagglutinin, streptolysin O) or for 7 days (mixed leukocyte culture and tumor cells). Harvesting was accomplished as previously described[11] by addition of 2 μCi of ^{3}H-thymidine with a specific activity of 1.9 Ci per millimole (Schwarz Bio Research, Orangeburg, New York) for 3 hours and measurement of the acid insoluble radioactivity by liquid scintillation counting.

Lymphocyte blastogenesis was measured as the net counts per minute (cpm) of tritiated thymidine incorporation per 1×10^6 cultured lymphocytes, with the thymidine incorporation of the appropriate controls subtracted from that of the stimulated cultures. A 100 per cent increase or 50 per cent decrease in thymidine incorporation compared to the appropriate control was significant stimulation or inhibition at the 95 per cent level of confidence[15] and a 300 per cent increase or 75 per cent decrease was significant at the 99 per cent level of confidence. The stimulation index was defined as the cpm of a stimulation culture divided by the cpm of the appropriate unstimulated lymphocyte culture.

Membrane immunofluorescence with the use of highly class-specific animal anti-human IgG, IgA, or IgM purified by absorption to an elution from insoluble immunoabsorbent antigens[16] was performed according to the method of Möller.[17] Leukemia cells were thawed rapidly, washed three times with modified Eagle's medium, and then incubated at room temperature for 60 minutes with either patient's serum, serum from a normal donor with the blood Type AB, rh positive, or McCoy's medium (modified) with 30 per cent fetal calf serum. The incubated cells were washed three times in phosphate-buffered saline with McCoy's medium before being exposed to the fluorescein-conjugated antiserums specific for human IgG, IgA, or IgM heavy chains as previously described.[18] Leukemia cells were resuspended in the appropriate fluorescein-conjugated antiserum and incubated for 30 minutes at 22°C. After 3 washings in phosphate-buffered saline with McCoy's medium, the cells were mounted in a solution of phosphate buffered saline and glycerin on glass slides and read immediately or fixed with gluteraldehyde and read within 7 days. A positive reaction was recorded when 20 per cent or more of the cells showed surface-membrane fluorescence with an antibody reagent against a single immunoglobulin class (100 cell count).

Of the 34 patients 24 had acute myelogenous leukemia, and 10 acute lymphoblastic leukemia. Their median age was 28 years (range, 15 to 71). There were 19 males and 15 females. The various regimens of intermittent chemotherapy were similar to those previously presented.[19] Nine patients were treated with cyclophosphamide, vincristine, arabinosyl cytosine, and prednisone, 13 with daunomycin, vincristine, arabinosyl cytosine, and prednisone, 7 with vincristine, arabinosyl cytosine and prednisone, 2 with arabinosyl cytosine and thioguanine, 1 with 6-mercaptopurine, vincristine, methotrexate, and prednisone, and 2 with guanazole.[20] All chemotherapy regimens consisted of 5 days of chemotherapy with intervals off therapy of at least 9 days. The patients were studied as soon as their peripheral blood was free of leukemic cells, just before a course of chemotherapy, and at least 5 days after the termination of the previous course, just before the 2d or 3d course of remission-induction therapy. Twenty-seven of the 34 patients had not previously been treated with chemotherapy, and 7 had relapsed from previous chemotherapeutic regimens. Criteria for remission were as previously described.[21]

Statistical Analysis

Statistical analysis was performed by the chi-square test.

Results

Nineteen of 24 patients with acute myelogenous and five of 10 with acute lymphoblastic leukemia had a positive blastogenic response to their leukemia cells in either autologous or allogenic serum. In nine patients who had a positive blastogenic response only in allogenic serum, this response was partially or completely abolished when the cultures contained the patient's own serum instead of allogenic serum. Eight of these patients had acute myelogenous leukemia. Four of these patients have been studied serially, and the inhibitory effect has been constant in three.

Results of the membrane immunofluorescence studies with leukemia cells are shown in Table 1. The cells of eight of 24 patients with acute myelogenous but none of 10 patients with acute lymphoblastic leukemia demonstrated immunofluorescence with anti-IgG antiserum when the cells were incubated either in McCoy's medium or in the patient's own serum. Morphologically, the fluorescent staining appeared as large, nonuniform clumps on the surface of the leukemia cells.

Table 1. Correlation between Membrane Immunofluorescence, Blastogenic Response, and Serum Inhibitory Effect.

Blastogenic Response*	Serum Inhibition	Membrane Immunofluorescence†			
		A		B	
		yes	*no*	*yes*	*no*
Yes	Yes	7‡	1	0	1
Yes	No	1	10	0	4
No	No	0	5	0	5
Totals		8	16	0	10

*Stimulation index ≥ 2.

†A represents patients with acute myelogenous, & B those with acute lymphoblastic leukemia.

‡Difference between immunofluorescence in group with serum inhibition & other 2 groups without inhibition highly significant (chi-square p value < 0.005).

The correlation between the blastogenic response, its serum inhibition and the presence of membrane immunofluorescence is also shown in Table 1. Seven of the nine patients with inhibition of blastogenesis by their own serum had positive immunofluorescence with anti-IgG antiserum. In contrast, only one of the 15 patients with a positive blastogenic response who did not have a serum inhibitory effect had a positive

immunofluorescence. Finally, cells from patients who did not have a blastogenic response to tumor cells failed to show this immunofluorescence reaction.

Table 2 lists laboratory and clinical data on the seven patients with serum inhibitory effect and positive membrane immunofluorescence. Four of them were males, and three were females. The median proportion of positive fluorescence cells was 40 per cent. The percentage of fluorescent cells did not increase after the cells were incubated in the patients' own serums as compared to incubation with McCoy's medium except in one case. Fifty per cent of the cells of one of these patients also reacted with anti-IgM and anti-IgA antiserums.

Table 2. Median Values* for Positive Immunofluorescence and Serum Inhibitory Effect.

Source of Serum	Positive Immuno-fluores-cence (IgG)	Blastogenic Response of Patient Lymphocytes			
		Tumor Cells		Phytohemagglutinin	
	%	cpm†	SI‡	cpm	SI
None	40 (5-50)	—	—	—	
Autol-ogous	40 (15-50)	0 (0-18.7)	0.5 (0.5-27)	48.4 (31.0-57.8)	56.1 (19.8-380.0)
Allogenic	—	4.6 (1.1-120.0)	14.8 (2.5-1250.0)	58.5 (41.7-107.1)	164.0 (23.2-771.0)

*Ranges in parentheses. †Per 10^5 lymphocytes × 10^3. ‡Stimulation index.

Five of the seven patients with inhibitory effect had complete abolition of the blastogenic response (both stimulation index and net cpm) when the leukemia cells were incubated in autologus serum, and the inhibitory response was over 80 per cent in the other two. In contrast, only two of the seven patients showed significant inhibition of the response to phytohemagglutinin by autologous serum, and in one the inhibitory effect was less than 70 per cent. The inhibition by phytohemagglutinin was noted only after calculation of the stimulation index but was not significant when the net cpm were considered. In these two cases, the baseline lymphocyte counts were higher in autologous serum than in allogeneic serum. Six of these seven patients achieved a complete remission with chemotherapy.

The correlation between the degree of blastogenic response to autologous leukemia cells and the clinical response of the 34 patients is shown in Table 3. Seventeen of 20 patients with a stimulation index greater than 3 achieved a remission, and 12 of 15 had acute myelogenous leukemia. In contrast, only nine of 14 patients with a stimulation index lower than 3 achieved remission. Also, 16 of 24 (67 per cent) of patients with acute myelogenous, as compared with only four of 10 (40 per cent) with acute lymphoblastic leukemia, had a stimulation index greater than 3.

Table 3. Correlation between Degree of Blastogenic Response* of Peripheral Leukocytes to Autologous Leukemia Cells and Clinical Response.

SI†	Remission‡			No Remission		
	Acute Myelog-enous Leukemia	Acute Lympho-blastic Leukemia	Total	Acute Myelog-enous Leukemia	Acute Lympho-blastic Leukemia	Total
	no. of patients					
< 3	5	4	9(64%)	3	2	5
> 3	13	4	17(85%)	3	0	3
> 5	12	3	15(94%)	1	0	1
>10	8	1	9(100%)	0	0	0

*Maximum response to unirradiated & irradiated cells in either autologous or allogenic serum.

†Stimulation index.

‡Includes complete remission (bone marrow with < 5% blast forms, & normal peripheral blood) & partial remission (bone marrow with 5-30% blast forms with no circulating blast forms in peripheral blood).

The correlation between the degree of blastogenic response to leukemia cells in terms of net cpm and the clinical response is shown in Table 4. The majority of patients with acute myelogenous but only two of 10 with acute lymphoblastic leukemia had cpm over 1000. However, there was no correlation between the degree of blastogenesis as measured by cpm and the clinical response of the patients.

Discussion

To acquire a more complete understanding of a patient's immune response to autologous tumor, it is important to consider both the cell-mediated and the humoral aspects of tumor immunity. This study of acute leukemia was designed to determine the inter-relation between lymphocyte blastogenic responses to leukemia cells, any serum effects on these responses and the presence or absence of immunoglobin on the leukemia cells. In addition to the knowledge gained, this type of combined approach should form a background for the design of rational immunotherapy programs in acute leukemia and other neoplasia.

The present study confirms previous observations that the lymphocytes of the majority of patients with acute leukemia can mount a blastogenic response to their own leukemia cells.[6,8] Although a correlation between lymphocyte responsiveness to autologous leukemia cells and the clinical status of the patients was not

Table 4. Correlation between Degree of Blastogenic Response* of Peripheral Leukocytes to Autologous Leukemia Cells and Clinical Response.

CPM	Remission		No Remission	
	Acute Myelog-enous Leukemia	Acute Lympho-blastic Leukemia	Acute Myelog-enous Leukemia	Acute Lympho-blastic Leukemia
<1000	5	6	1	2
>1000	13	2	5	0
>5000	8	0	4	0

*Maximum response to unirradiated & irradiated cells in either autologous or allogenic serum.

reported in these earlier studies, the current studies demonstrate a strong correlation between the degree of blastogenic response to leukemia cells and the response to treatment — e.g., the higher the stimulation index, the greater the chance for a chemotherapy-induced remission. We have previously demonstrated that one can relate prognosis of the patient with acute leukemia to the degree of general immunocompetence.[19]

The incomplete correlation between the stimulation index and blastogenic response as determined by net counts per minute (Table 4) emphasizes the importance of examining both measurements of lymphocyte blastogenic data. Many of the patients with high net counts per minute had low stimulation indexes because the unstimulated lymphocyte counts were high. Several of these patients failed to respond to chemotherapy. Possibly, the blood was not yet free of leukemic cells, and the thymidine uptake of the unstimulated control cultures represented the activity of both normal lymphocytes and leukemic blast forms (despite morphologic absence of blast forms).

A point of debate is whether a blastogenic response to autologous leukemia cells represents a primary response in vitro or whether it actually represents established immunity.[6] The fact that we could correlate the degree of blastogenic response with the clinical response and with evidence of antibody tends to support the idea that blastogenic responses to leukemia cells may indeed represent specific tumor immunity.

Earlier studies of humoral responses to human leukemia cells have detected immunoglobulin on the cell surfaces only rarely. The current demonstration that positive membrane immunofluorescence occurred as frequently when the cells were incubated in McCoy's medium as in the patients' own serums suggests that immunoglobulin is already coated on the cells. Whether the cells themselves are producing the IgG immunoglobulin (as Burkitt-lymphoma cells produce IgM[22]) or are being coated from the outside, as described for the IgG on some Burkitt cells,[23] cannot be determined from these studies.

The idea that the positive fluorescence represents bound immunoglobulin in our group of patients is substantiated by the morphologic appearance of the immunoglobulin on the tumor cells. Immunoglobulin receptors on cells of B origin show either a fine, uniform ring pattern of fluorescence or cap formation.[24] In contrast, the immunoglobulin on the leukemia cells in this study appeared as nonuniform, larger clumps of staining, characteristic of bound immunoglobulin.

Only patients with acute myelogenous leukemia had evidence for bound immunoglobulin. Thus, either patients with the acute lymphoblastic type do not make such antibody or, perhaps, their leukemia cells are not very immunogenic, as suggested by the greater autologous blastogenic response to acute myelogenous than to acute lymphoblastic cells. This response might also be due to diminished reactivity on the part of patients with acute lymphoblastic leukemia; the fact that cells of these patients do not appear to produce surface immunoglobulin as do tumor cells from other lymphoid tumors such as chronic lymphocytic leukemia[25] or malignant lymphoma[26] suggests the cells of acute lymphoblastic leukemia may be of thymic origin, may not have differentiated sufficiently to produce immunoglobulin or may not be of lymphoid origin.

It has previously been recognized that patients with cancer may have factors in their serum that inhibit cell-mediated immune responses to various mitogens and tumor cells.[9] The Hellströms and their co-workers have demonstrated a tumor-specific blocking or enhancing antibody in the serum of many patients with progressively growing solid tumors.[10] Mavligit[27] and Vánky[28] and their colleagues have demonstrated serum inhibition of blastogenic reactivity to tumor cells in patients with a variety of solid tumors. Initial studies by Sjögren et al. suggested that the blocking factor may be tumor antigen-antibody complex.[29] Although, our present study shows a strong correlation between the presence of immunoglobulin on leukemia cells and the blocking of the blastogenic response, it does not clarify whether antibody, antigen-antibody complexes, or perhaps other factors may be involved with the inhibitory phenomenon. Immunoglobulin was already present on the leukemia cells, and the number of positive cells did not increase after incubation in autologous serum. Thus, it appears that autologous serum may contain a substance or substances, such as soluble antigen, that interact with bound immunoglobulin to account for the fact that inhibition of blastogenesis was noted only in autologous serum but not in allogenic serum.

In our study, the presence of immunoglobulin on leukemia cells and the presence of inhibitory effect on the blastogenic response was correlated with a good prognosis. Six of the seven patients with this constellation of findings achieved a clinical remission. Five of these patients remained in remission three to 12 months later. The serum inhibitory effect has been consistent throughout the clinical course in all but one patient.

Our results further support the importance of complete immunologic evaluation of patients with leukemia. In this way we can define more accurately the major immunologic factors in the control of the cancer. For example, if we can demonstrate that humoral antibody is of benefit to patients with leukemia consideration should be given to the potential use of passive immunotherapy (serotherapy). However, if the presence of antibody is a detrimental factor to effective killer lymphocyte activity, perhaps intensive plasmapheresis would be indicated. Since there is a strong correlation between the degree of blastogenic response to leukemia cells and a clinical remission we should also consider attempting to increase the blastogenic response by immunization with tumor cells[7] or adjuvants of immunotherapy such as BCG[30] to evaluate the immunologic effect, independently of any therapeutic effect.

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IMMUNOGLOBULIN ON TUMOR CELLS AND TUMOR-INDUCED LYMPHOCYTE BLASTOGENESIS IN HUMAN ACUTE LEUKEMIA

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Revision by Barbara J. Culliton

THE immune system appears to play an important role in determining how a patient will respond to a tumor. Presumably, he has a better chance of survival or, at least, of experiencing remission, if his immune system is alert to the presence of tumor cells. We are not yet certain, however, of the precise mechanism or mechanisms involved in natural tumor immunity. Nor do we fully understand the facets of immune responsiveness that may vary specifically from one type of tumor to another.

During the last several years, there has been increasing evidence that tumor-associated antigens are present in a variety of human neoplasms, including acute leukemia. Concurrently, emphasis has been placed on the cell-mediated lymphocyte response to these antigen-bearing tumors as a measure of immune reactivity. Previously, we showed a correlation between a patient's prognosis and his general level of immunocompetence.* In this study, we are extending that correlation to more specific areas of immunocompetence. And we are suggesting that emphasis should be placed not only on cell-mediated, but also on humoral, or circulating antibody response in achieving some measure of natural tumor immunity, particularly with regard to acute leukemia.

Thus, to acquire a more complete understanding of

*For specific references, see preceding article.

a cancer patient's immune response to his own tumor, it is important to consider both the cell-mediated and the humoral aspects of tumor immunity. Our study of 34 adults with acute leukemia was designed to determine the inter-relation of a lymphocyte blastogenic response to leukemia cells (a response that indicates lymphocyte recognition of tumor antigen), any effects the patient's own serum has on this response, and the presence or absence of immunoglobulin on the surface of the tumor cells.

Results*

Nineteen of the 24 patients with acute myelogenous leukemia and five of the 10 with acute lymphoblastic leukemia had a positive blastogenic response to their tumor cells stimulated in either their own or someone else's serum. Blastogenesis observed when the serum of normal donors was used was partially or completely abolished in eight of the positive cases of acute myelogenous leukemia when the cultures contained the patient's own serum instead. One individual with acute lymphoblastic leukemia showed inhibition of the blastogenic response with autologous serum as well.

Membrane immunofluorescence analysis revealed immunoglobulin on the tumor cell surface in eight of the 24 patients with acute myelogenous leukemia but in none of the 10 persons with acute lymphoblastic disease. We used anti-IgG antiserum and were able to detect antibody when the cells were incubated in McCoy's medium (which contains no human blood products) or in autologous serum. The median proportion of cells coated with immunoglobulin was 40 per cent. In one case, 50 per cent of a patient's cells reacted positively with anti-IgM and anti-IgA antiserums. The other seven patients reacted exclusively to anti-IgG antiserum.

When we looked for cases in which there was both inhibition of blastogenic response and evidence of immunoglobulin, we found seven. Only one individual of 15 who had a positive blastogenic response that was not inhibited by serum had any sign of antibody by the immunofluorescence test. Cells from patients who did not have any blastogenic response consistently failed to show evidence of antibody.

Thus, of the total of 34 patients studied, seven showed both blastogenic inhibition and positive immunofluorescence. Five of the seven had complete abolition of blastogenic response in autologous serum as measured both by the stimulation index and by net counts per minute. The inhibitory response was 80 per cent in the other two cases. Six of these seven patients achieved complete remission with chemotherapy.

There appears to be a direct correlation between the degree of blastogenic response to autologous leukemia cells and clinical status. Twenty of the total group of 34 patients had a stimulation index greater than three. Seventeen of them achieved at least temporary remission. Only nine of the remaining 14 whose stimulation index was less than three went into remission. Comparing the 24 persons with acute myelogenous leukemia to the 10 with acute lymphoblastic leukemia, we found that 16 of 24 or 67 per cent of the acute myelogenous leukemia group had a stimulation index greater than three but that only four of 10 or 40 per cent of the acute lymphocyte leukemia patients showed such a strong response.

*Materials and Methods as described in preceding article, pages 169-170. It is to be noted that the incorporation of tritiated thymidine in cultured lymphocytes was used to assess the blastogenic response.

Discussion

Our results support the view that one can correlate specific features of the cell-mediated and humoral immune systems with prognosis in acute leukemia. Generally, the correlation we find pertains to individuals with acute myelogenous leukemia rather than to those with acute lymphoblastic leukemia.

Our results also confirm previous observations that a majority of patients have lymphocytes capable of mounting a blastogenic response to their own leukemia cells. Other evidence of cell-mediated immunity has been found by Oren and Herberman, who reported in 1971 that the majority of patients with acute leukemia whom they studied had positive skin tests to membrane preparations of their own leukemia cells. It has also been shown that in some patients with acute leukemia, autologous leukemia cells stimulate lymphocyte blastogenesis and cytotoxic changes in lymphocytes in vitro.

No correlation between lymphocyte responsiveness to autologous leukemia cells and the clinical status of the patient was reported in these earlier studies. Now, the current experiments reveal what we had suspected: there is a strong correlation between the degree of blastogenic response to leukemia cells and the response to treatment which tells us that individuals whose cells can react to tumor-associated antigen to a significant degree have the greatest chance of a drug-induced remission.

A point of debate is whether this blastogenic response to autologous leukemia cells represents a primary response in vitro during the week-long period of incubation, or whether it actually represents established immunity in vivo. The fact that we could correlate the degree of blastogenic response with clinical response to therapy and the presence of antibody bound to acute myelogous leukemia cells tends to support the premise that blastogenic responses to leukemia cells may indeed be an expression of a specific tumor immunity that exists in vivo.

If this endogenously induced cell-mediated immunity exists as it appears to, why does it fail to control clinical cancer? One possible explanation of this unhappy situation is that substances in the serum interfere with cell-mediated immunity. The Hellströms and their coworkers, for example, have described a factor in the serum of patients with solid tumors that appears to block the path of killer lymphocytes (i.e., lymphocytes that can produce cytotoxic changes in tumor cell cul-

tures). This factor is known both as blocking antibody and as enhancing antibody.

Earlier studies of humoral responses to human leukemia cells have only rarely detected immunoglobulin on the cell surface. The work we are reporting here suggests that immunoglobulin is, in fact, a common feature on the surface of certain types of leukemia cells, and of acute myelogenous leukemia in particular. Our demonstration that positive membrane immunofluorescence occurs as frequently when the cells are incubated in McCoy's medium, as in the patient's own serum, leads us to think that immunoglobulin is already coated on the cells. In our tests, we identified immunoglobulin G (IgG) on acute myelogenous leukemia cells in some cases. Whether the cells themselves are producing IgG (as Burkitt-lymphoma cells produce IgM) or are being coated from the outside as described for the IgG that is found on some Burkitt cells cannot be resolved from the present experiments.

The idea that positive membrane fluorescence represents immunoglobulin bound to the cell surface is substantiated by the morphologic appearance of the IgG on the tumor cells. The immunoglobulin showed up as nonuniform, large clumps, an appearance characteristic of bound immunoglobulin.

Only patients with acute myelogenous leukemia had detectable immunoglobulin. In 10 out of 10 cases of individuals with acute lymphoblastic leukemia, we saw no signs of antibody on the leukemic cell surface. There are a number of possible explanations. One is that acute lymphocytic leukemia cells are only weakly antigenic, as suggested by the fact that the strongest blastogenic responses we recorded were to acute myelogenous leukemia cells, not the acute lymphatic leukemia cells. It may also be because of a generally diminished immune reactivity among patients with acute lymphoblastic leukemia. The fact that cells of these patients do not appear to produce surface immunoglobulin as do tumor cells from other lymphoid tumors including chronic lymphocytic leukemia or malignant lymphoma suggests the cells may be of thymic origin or may not have differentiated sufficiently to produce immunoglobulin. In any case, it is clear from these and other studies that there are distinct and, probably, clinically significant immunologic differences among leukemia cells of various types.

Clinical remission of disease occurred in six of seven of our patients in whom we found immunoglobulin bound to their leukemia cells and whose serum inhibited blastogenesis. Five of these individuals are in remission three to 12 months after the initial study and the ability of serum to block blastogenesis has been consistent throughout this time in all but one patient.

We thus correlate a good prognosis in individuals with acute myelogenous leukemia under seemingly contradictory circumstances. A positive blastogenic response is a good sign because it means that the patient's lymphocytes have recognized the tumor antigen. On the other hand, if it can be inhibited by autologous serum from patients whose leukemia cells are coated with immunoglobulin, that too is favorable. The key appears to be in the patient's own serum. Our experiments do not tell us whether antibody, antigen-antibody complexes or, perhaps, other factors in the autologous serum are involved in the inhibitory phenomenon. Immunoglobulin apparently is present on the leukemia cells from the start; the number of immunoglobulin coated cells did not increase after incubation with the patient's own serum. Therefore, it is reasonable to think that autologous serum may contain some substance or substances, such as soluble antigen, that interact with the bound immunoglobulin. This would explain why blastogenesis is inhibited in autologous serum from patients with immunoglobulin coated leukemia cells but not from allogenic serum from healthy donors.

Our experience with these three measures of the immune status of patients with acute leukemia supports the importance of regular immunologic evaluation of these individuals. In this way we believe we can define more accurately the major immunologic factors in the control of cancer. For example, if we can demonstrate that humoral antibody is of benefit to patients with leukemia, we would consider the use of passive immunotherapy (serotherapy) in their treatment. However, if we see that antibody is getting in the way of killer lymphocytes that might otherwise destroy a tumor, we would contemplate intensive plasmapheresis. Because there is a strong correlation between the degree of blastogenic response to leukemia cells and a clinical remission, we are also thinking about trying to increase the blastogenic response by immunizing patients with tumor cells or with adjuvants such as BCG in order to evaluate the immunologic effect of immunization independently of its therapeutic effect.

It is becoming apparent that appropriate therapeutic steps in one situation might well be inappropriate in others, and we have to learn to tell them apart. It is our conviction that by studying immune status of patients by using the three measures we have described, we will come closer to acquiring the information we need.

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TWIN BILL ON TUMOR IMMUNITY

Readers who discover that the first and second articles in this issue bear identical titles will probably think, "Oh-oh – they've goofed again." Well, the twin reports may be a blunder, but not of the usual unintentional variety.

For the uninitiated, immunology, and particularly tumor immunology, tends to be a difficult subject. And things become no easier when some immunologic phenomena of leukemia are described in terms of lymphocytic blastogenesis, membrane immunofluorescence, and abrogation of positive blastogenesis by autologous but not by allogeneic serum. Nor is the picture clarified by responses that reflect a mixed bag of partly cell-mediated immunity, and partly humoral immunity with enhancing or blocking antibodies or antibody-antigen complexes.

Isaac Asimov* tells us that in the future scientists will still be doing their own experiments (which is reassuring), but that their reports will be prepared by science writers. Many others have argued that the generally dull and tacky prose of the physician reporter should be given luster and fluidity by the professional journalist. So, as an experiment, Barbara Culliton, who writes for the News and Comment section of Science, was persuaded to see if she could recast the complexities of the Gutterman report into words at once more palatable and more comprehensible. In the development of this project, Dr. Gutterman and his associates were not only co-operative, but also enthusiastic.

The transformation, it turns out, is far from easy. In the first place, although the style of the second paper is entirely Culliton's she was asked to confine herself – in the interest of permitting a valid comparison – to the ideas expressed in the first paper, and to avoid the use of additional information. Under such conditions, a science writer is more of a translator than an independent author. Secondly, the section on methods – a section that in the *Journal* is as a matter of course already presented in an abbreviated and simplified form – does not lend itself to rewriting. Thirdly, the science writer can hardly be expected to present a crystal-clear logic culminating in some readily understood and essentially simple conclusion when the nature of the subject investigated and the findings themselves are so complex as to preclude the formulation of any sharply defined concepts. Finally, in spite of the best of intentions, authors, science writer and editor do not necessarily agree on the best way of expressing an idea.

The decision whether or not Culliton made Gutterman easier is the reader's. Does this pilot test warrant any reruns? Is the rewrite worth the effort and expense involved?

F.J. Ingelfinger, M.D.

*Asimov I: Future of medical communication, Doctors and People Talking: Addresses in the Lowell Institute Lecture Series. Boston, Boston University Medical Center, 1972, pp 132-141

RESERVOIRS FOR INTRAVENTRICULAR CHEMOTHERAPY

In recent years it has become increasingly popular to place plastic reservoirs of the type described by Ratcheson and Ommaya[1] beneath the scalp with a cannula running from the reservoir to the lateral cerebral ventricle through a burr hole in the skull. Such a device allows easy access to cerebrospinal fluid for sampling, and for injection of drugs that do not cross the blood-brain barrier. At least one group is placing such subcutaneous reservoirs routinely for prophylactic treatment of the central nervous system in acute leukemia,[2] and at Memorial–Sloan Kettering Cancer Center, subcutaneous reservoirs are placed in all patients with established meningeal leukemia. In this issue of the *Journal*, Diamond and Bennett report their experience with the use of such reservoirs for the treatment of patients with fungal meningitis. In only 16 of their 21 patients did the reservoirs finally function well, and of these, 11 had complications that interfered with therapy at some point in their course. The authors, however, point out that such a high complication rate has not been the experience of others who have placed reservoirs for the treatment of other diseases, and it has not been our experience in patients with meningeal leukemia.

The advantages of subcutaneous reservoirs are several. For one thing, drugs are easily administered into the cerebrospinal fluid, and samples of ventricular fluid are easy to collect. The injection through the scalp into the reservoir can be performed with a small needle, usually a No. 23 or No. 25 butterfly type, and the procedure is virtually painless, in contradistinction to the current mode of intrathecal therapy via lumbar or cisternal puncture. Another advantage is that if the reservoir is functioning, there is certainty about location of the injected drug. Larson and his colleagues[3] have indicated that about 10 per cent of intrathecal injections via lumbar puncture fail, the material being placed into the subdural or epidural space instead of the subarachnoid space. Furthermore, the physician is never certain where he is placing medication at lumbar-puncture since there is no bedside method of distinguishing puncture of a subdural sac from that of the subarachnoid space. Finally, the drug is probably more widely distributed when injected intraventricularly than when injected via the lumbar route. Isotope studies suggest that, in the absence of communicating hydrocephalus, isotope injected into the lumbar subarachnoid space does not reliably enter the cerebral ventricles in appreciable quantities but instead rises over the convexities of the brain only.[4] By contrast, drugs injected into the lateral ventricles follow the normal flow of spinal fluid and wash the entire subarachnoid space.[5]

Many of the disadvantages of this mode of therapy have been detailed by Diamond and Bennett. Some of these disadvantages can be avoided by attention to technic. For example, placing the reservoir after pneumoencephalography allows x-ray control of localization of the tip of the catheter and would have prevented the six misplaced catheters that the authors report. Performing ventriculosystemic shunts along with reservoir placement probably, as the authors suggest, makes intraventricular injection of drugs useless since the drugs leave the cerebrospinal fluid by the shunt almost as soon as they are injected. Furthermore, the shunting procedure itself may have been responsible for many of the neurologic sequelae attributed to reservoir placement. Plugging of the catheter, often by proteinaceous material, is the most common reason for reservoir failure but is probably more common in fungal meningitis, in which ventricular protein concentration is much higher than in leukemia or meningeal cancer.

One complication that the authors do not detail is that of drug toxicity. Drugs that appear relatively safe when given into the lumbar subarachnoid space may be more toxic when given into the cerebral ventricles. In a case reported by Kress and Cantrell[6] hemiplegia developed after intraventricular injection of amphotericin B, and two groups describe leukoencephalopathy produced by methotrexate given intraventricularly.[7,8] Both these drugs appear to be safer when given in the lumbar sac, although neurologic complications occasionally follow lumbar injection of either.

Our current experience at the Memorial–Sloan Kettering Cancer Center is too limited to recommend routine use of reservoirs for intrathecal chemotherapy, particularly since the full range of drug toxicity is unknown. The advantages of subcutaneous reservoirs, however, are sufficiently attractive to warrant further trial, particularly in centers where close neurologic and neurosurgical scrutiny of possible complications is available.

Jerome B. Posner, M.D.

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THE MONOCYTE: NEW CONCEPTS OF FUNCTION

Blood monocytes are usually thought of as a minor subgroup of circulating phagocytic leukocytes that