# CELLULAR IMMUNE REACTIONS IN HUMAN ACUTE LEUKEMIA

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Cellular immune reactions are generally thought to play a major role in host resistance against tumor growth. Human acute leukemia cells have been found to contain tumor associated antigens, and it is possible to measure the cell-mediated immune response to these antigens. In addition to specific reactivity, it is quite important to evaluate the functional integrity of the cellular immune system in leukemia patients. The disease process itself or the chemotherapeutic agents could cause general depression in reactivity. The techniques currently being used for such studies are summarized in Table 1. In this paper, we will review the information available from each of these approaches, and discuss their possible relevance to the clinical state.

**Table 1. Assays of Cellular Immunity in Human Acute Leukemia**

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|---|
| Assays of competence of cellular immunity system |
| 1) Delayed hypersensitivity skin reactions: standard recall antigens and ability to be sensitized to DNCB, KLH |
| 2) Lymphocyte stimulation by mitogens (e. g. PHA), common antigens and allogeneic leukocytes |
| 3) Enumeration of thymus-derived lymphocytes (T cells) and bone-marrow derived lymphocytes (B cells) in peripheral blood |
| 4) Cytotoxic reactivity of lymphocytes against human lymphoid cell lines |
| Cell-mediated immunity against leukemia associated antigens |
| 1) Delayed hypersensitivity to membrane and soluble extracts of blast cells |
| 2) Lymphocyte stimulation by autologous blast cells and soluble extracts |
| 3) Cytotoxic reactions against blast cells |
| 4) Inhibition of leukocyte migration by extracts of blast cells |

## Competence of Cellular Immunity System

Three aspects of the competence of the cellular immunity system in acute leukemia patients are of interest: a) the effect of the tumor burden on reactivity; b) the effect of chemotherapy; and c) the inherent ability of patients to have cell-mediated immunological reactions against a variety of antigens. Unfortunately, it is difficult to clearly study each of these factors. The ideal time to obtain information on the patient's inherent reactivity would be prior to development of disease. Based on the theory of immunological surveillance (1, 2), one might anticipate that individuals developing leukemia have depressed immunological competence. However, it is not possible to obtain information at this time. It is practical to examine patients at the time of diagnosis, when tumor is present, and during clinical remission. Since chemotherapeutic agents are used to induce remission, their effects must also be considered.

Skin tests for delayed hypersensitivity have been extensively used to study cellular immunity in acute leukemia patients. In studies before thereapy (3) and shortly after the onset of therapy (4), decreased reactivity of some patients to standard recall skin test antigens was found. Hersh et al (4) found that depressed skin reactivity was associated with poor response to therapy for six months or longer had even less reactivity than that seen in initial studies (5). Chemotherapy may have had an important influence on these results. Borella and Webster (6), in a study of children with acute leukemia in remission, observed that long-term combination chemotherapy had immunosuppressive effects on skin test reactivity. Some treatment protocols appeared to be more immunosuppressive than others. Many of the patients of Hersh et al (4, 5) received COAP, which was noted to be very immunosuppressive.

Our laboratory also studied the response of ALL (acute lymphocytic leukemia) and AML (acute myelogenous leukemia) patients to a battery of standard recall antigens (7). We performed almost all of our tests after induction of remission, or at the time of bone marrow relapse. Patients at the National Cancer Institute are usually treated with combination hemotherapy, with repeated cycles of one week of treatment followed by two–three weeks off. To reduce the possible effects of treatment on the results, the patients were usually skin tested just prior to a course of therapy. Table 2 gives a summary of our data. Ninety-six percent of patients with ALL and all of the patients with AML reacted with at least one of the antigens, when tested in remission or in relapse. There were no significant correlations between the incidence of reactivity to any one of the particular antigens and clinical state, time of test in relation to chemotherapy, or length of survival. The reactivity of the ALL patients to PPD was high, due to immunotherapy with BCG. There are several possible explanations for the differences between our results and those of Hersh's group. Since they found that patients with intact skin reactivity were more likely to go into remission (4), it is possible that our patients, initially tested in remission, were a selected population. The type of chemotherapy used, and the timing of tests in relation to therapy, may also have been important differences.

Stimulation of patients' lymphocytes by mitogens, recall antigens and allogeneic leukocytes is another widely used technique for assessing immune competence.

Table 2. Skin Reactions to Recall Antigens

| Patients | PPD+ | % Positive Tests Mumps | Candida | Tricho. | SKSD++ | % Anergic |
|---|---|---|---|---|---|---|
| ALL,** remission | 36 | 67 | 42 | 14 | 78 | 4 |
| ALL, relapse | 45 | 92 | 67 | 0 | 86 | 8 |
| AML++ remission | 13 | 83 | 33* | 0* | 72 | 0 |
| AML, relapse | 15 | 88 | 31* | 0* | 45 | 0 |
| Normal | 20 | 84 | 71 | 31 | 89 | 0 |

* Significantly less reactivity than in normal controls, $p<0.05$.
\+ Purified protein derivative of tuberculin, intermediate strength.
++ Streptokinase, 40 units, streptodornase 10 units.
** Acute lymphocytic leukemia.
++ Acute myelogenous leukemia.

Hersh et al (4) found that one-third of leukemia patients studied had decreased responses to phytohemagglutinin and streptolysin O. As with the skin tests, most of these poorly reactive patients did not respond well to therapy. It has been found the time of testing, in relation to chemotherapy, has an important influence on the results (8, 9, 10). Reactivity was greatest 10–20 days after cessation of therapy, and in fact was sometimes higher than normal reactivity at this time.

Techniques have recently been developed which may allow enumeration of thymus-derived lymphocytes (T cells) and bone marrow-derived lymphocytes (B cells) in the peripheral blood. T cells ,appear to have receptors for sheep erythrocytes (E), while B cells have receptors for the third component of complement and can thereby bind E coated with antibody and complement (EAC). Rosette assays based on binding of E and EAC are easy to perform and may provide useful information in cancer patients. Many cancer patients have decreased percentages of circulating T cells (11, 12). Sen and Borella (13) have found that longterm chemotherapy caused depression in lymphocyte counts, and B cells were decreased more than T cells. After cessation of therapy, the B cells returned to normal levels within two to three months, whereas recovery of T cells took longer. No systematic serial study of these cell populations at different phases of disease have been reported as yet.

Rosenberg et al (14) have recently found that the lymphocytes of most normal individuals have cytotoxic reactivity against human lymphoid cell lines. McCoy et al (15) found that many patients with solid tumors and patients with immune deficiency diseases had significantly reduced activity. Fig. 1 shows the results with leukemia and lymphoma. Many of the leukemia patients had low reactivity. Reactivity was found to vary at different times in relation to chemotherapy (16). However, no consistent pattern was seen among different patients. Patients who were off therapy for four to eight weeks (points labelled Rx in Fig. 1) had normal or high reactivity.

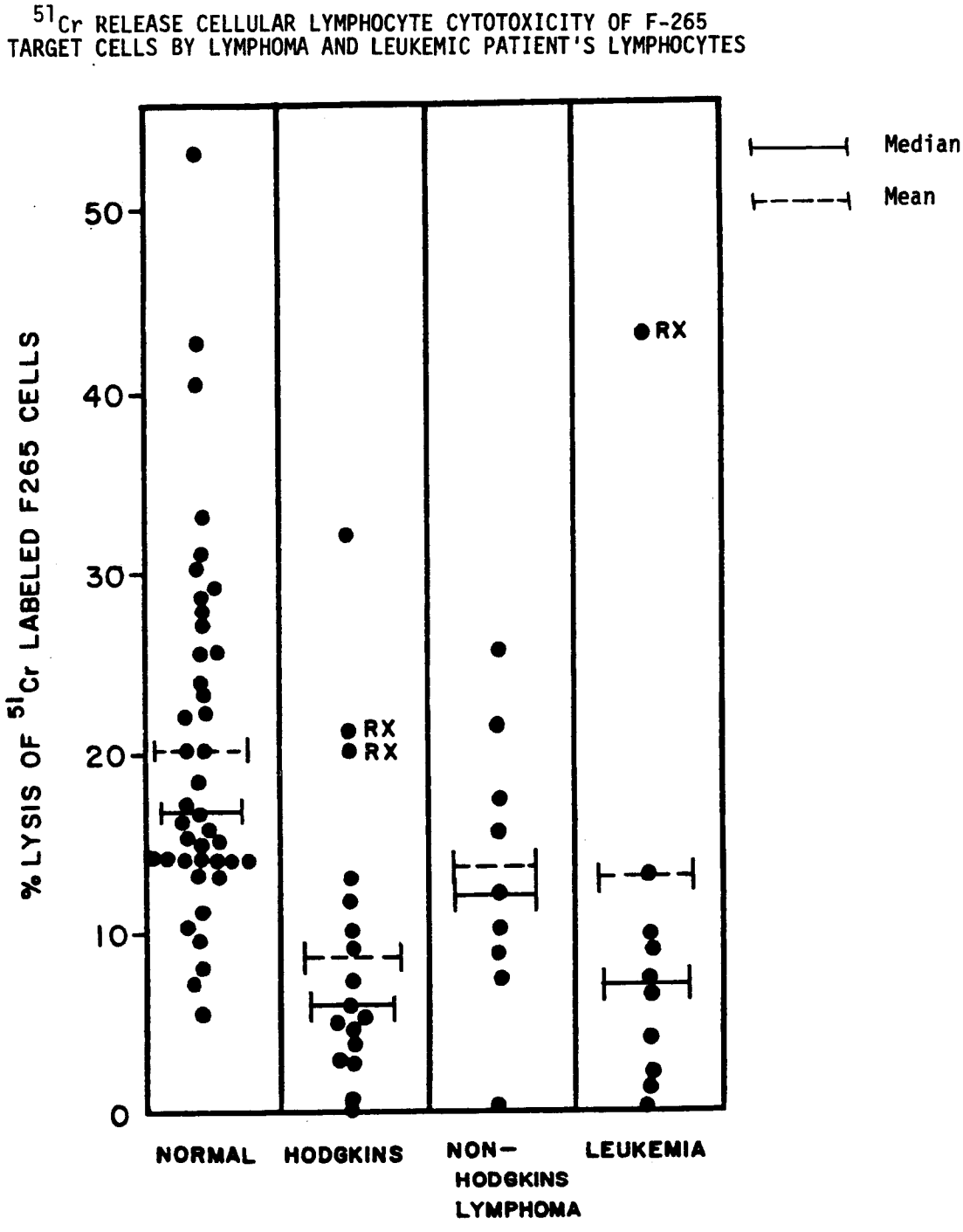


Fig. 1

## Cell-Mediated Immunity Against Leukemia Associated Antigens

Skin testing with membrane or soluble extracts of blast cells has been used to measure delayed hypersensitivity reactions to leukemia associated antigens (7, 17). Table 3 is a summary of our tests with autologous membrane extracts of blast and remission cells (7). In patients with ALL who were in remission, positive reactions to autologous blast extracts were obtained in 20 of 44 tests. ALL patients tested in

relapse gave only one positive reaction. In AML, there was also a significant correlation of reactivity to blast extracts and clinical state. Serial skin tests were performed in 29 ALL and 8 AML patients (7). In most cases, reactivity to autologous blast cell extracts fluctuated in parallel with changes in clinical state. Patients who had positive reactions in remission generally became unreactive at the time of relapse. This represented specific depression, in that reactions to recall antigens did not vary significantly.

In contrast to our results with autologous membrane extracts, it has recently been reported that no positive reactions of acute leukemia patients were elicited by autologous viable cells (17) or mitomycin-C treated blast cells (3). Gutterman et al (17) did find, however, that 3M potassium chloride extracted soluble antigen produced positive skin reactions.

These studies indicated that the form of the antigen used for skin testing may influence the results. Our group has performed studies on antigens solubilized and separated from AML cells (18), as indicated in Figure 2. Skin reactive antigens could be solubilized by low frequency sonication, and then separated by Sephadex G-200 and DEAE-cellulose chromatography. Table 4 gives the representative results of testing a patient with autologous DEAE fractions. Skin reactions were elicited by two of the fractions from blast cells, and not by the comparable fractions from remission cells. It has been possible to further separate the AML skin reactive antigens, by gradient acrylamide gel electrophoresis. Tabel 5 shows a test, in which only one region of the gel containing the blast extract gave a positive reaction. No reactions were elicited by the comparable remission cell fractions.

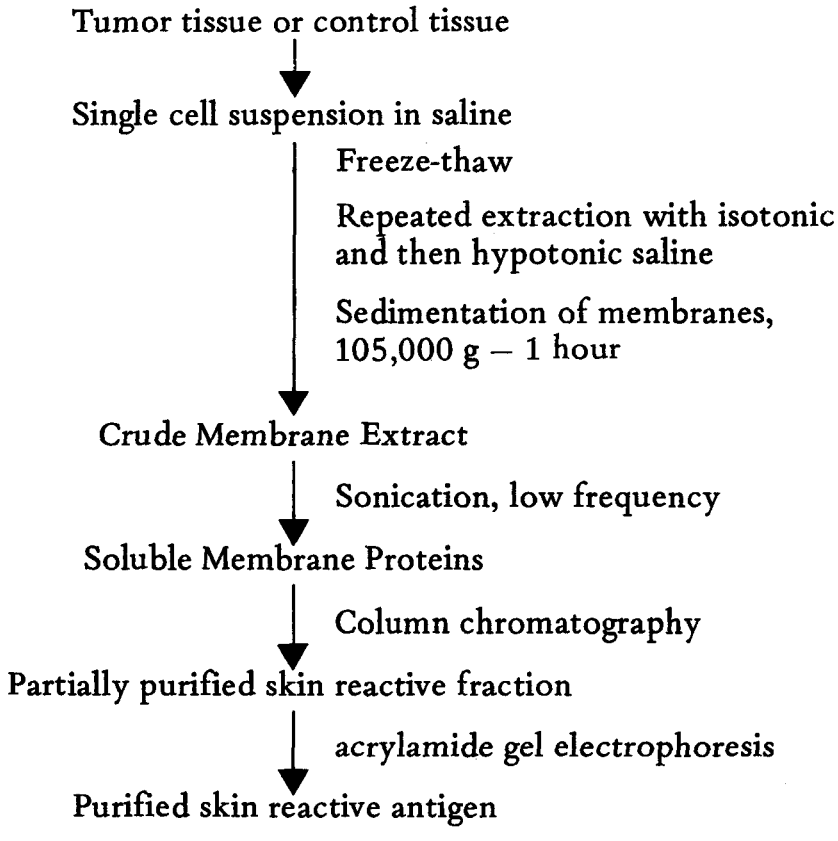


Fig. 2. Extraction procedure for solubilization and separation of skin reactive antigens on cells from patients with acute myelogenous leukemia.

Similar studies are now in progress with extracts of ALL cells and of fetal thymus cells. Some positive results have been obtained with the fetal thymus cells, which are of particular interest, since ALL blast cells appear to be T cells (12).

We have recently also tested patients with membrane extracts of cells from allogeneic leukemia patients (7). The results are summarized in Table 6. Positive reactions were observed in both AML and ALL patients, in response to extracts of blast cells from the same disease. Some of the extracts produced as much reactivity as the autologous extracts, while others were inactive. As with autologous extracts, reactivity to the allogeneic preparations correlated with disease status. The antigens detected on the allogeneic extracts appeared to be tumor associated, since extracts of remission cells or of normal leukocytes were unreactive.

Membrane extracts of human lymphoid cell lines have also given positive reactions in some patients with acute leukemia or lymphoma (19). Most of the cell lines were derived from Burkitt's lymphoma. As a control, patients were also tested with F-265, a cell line derived from normal lymphocytes. The results are summarized in Table 7. Patients with leukemia and lymphoma appear to be sensitized against an antigen on the tumor derived cell lines, whereas carcinoma

**Table 3. Skin reactions to Autologous Membrane Extracts**

| | Number of Positive Reactions/Total Tests (%+) | |
|---|---|---|
| Patients | Blast Cell Extract | Remission Cell Extract |
| ALL,* remission | 20/44 (45) | 0/14 (0) |
| ALL, relapse | 1/15 (7) | 0/5 (0) |
| AML,+ remission | 16/19 (84) | 0/5 (0) |
| AML, relapse | 9/18 (50) | 0/4 (0) |
| Normal | | 1/20 (5) |

* Acute lymphocytic leukemia.
+ Acute myelogenous leukemia.

**Table 4. Skin Tests with DEAE-Cellulose Fractions of Soluble Autologous AML* Cells (Herberman, Char, and Hollinshead, 1973)**

| Material Tested | DEAE Fraction | Skin Test Results (mm. induration) |
|---|---|---|
| Blast Cells | b | – (2) |
| Sephadex fraction | c | + (6. 9) |
| | d | + (6. 0) |
| Remission cells, | b | – (0) |
| Sephadex fraction | c | – (0) |
| | d | – (0) |

* Acute myelogenous leukemia

Table 5. Skin Tests with Acrylamide Gel Fractions (Herberman, Char, and Hollinshead, 1973)

| Material Tested | Gel Region | Skin Test Results |
|---|---|---|
| Blast Cells – | 1 | – |
| DEAE fractions c + d | 2 | – |
| | 3 | + |
| Remission Cells – | 1 | – |
| DEAE fractions c + d | 2 | – |
| | 3 | – |

Table 6. Skin Reactions to Allogeneic Membrane Extracts

| Patients | Extract | Number Positive/ Total % Positive |
|---|---|---|
| ALL*, remission | ALL blasts | 51/186 (27) |
| ALL, relapse | ALL blasts | 1/33 (3) |
| ALL, remission | ALL remission cells | 0/10 (0) |
| ALL, remission | AML blasts | 0/8 (0) |
| ALL, remission | Normal leukocytes | 0/20 (0) |
| AML+, remission | AML blasts | 4/7 (57) |
| AML, remission | AML remission cells | 0/5 (0) |
| AML, remission | ALL blasts | 0/6 (0) |
| AML, remission | ALL remission cells | 0/3 (0) |
| AML, remission | Normal leukocytes | 0/5 (0) |
| Normal | Normal leukocytes | 1/60 (2) |

*Acute lymphocytic leukemia.
+Acute myelogenous leukemia.

patients were unreactive. Reactivity in the ALL patients has correlated well with clinical state. Thus far, only patients in remission have been reactive.

Several groups have found that remission lymphocytes of patients with acute leukemia could be stimulated by autologous blast cells (10, 20–23). As with mitogens and common antigens, stimulation by autologous blasts was found to correlate with the length of time after cessation of a course of chemotherapy (10, 16). Autologous blast cells generally produced maximum stimulation at 10–20 days after chemotherapy, but the peak response to blast cells did not always occur at the same time as the peak response to mitogens or allogeneic leukocytes (16). Positive stimulation has also been found to correlate with good prognosis (23, 24).

Soluble extracts, prepared by treatment of blast cells with 3M potassium chloride, have also been shown to stimulate the autologous lymphocytes (17). We plan to perform similar studies with the antigens solubilized by sonication.

Table 7. Skin Reactions to Extracts of Lymphoid Cell Lines

| Patients | Tests Positive/Total Tests (%+) | |
|---|---|---|
| | Tumor Derived Cell Lines | F–265 |
| ALL* | 9/31 (29) | 0/21 (0) |
| CLL+ | 2/2 (100) | 0/1 (0) |
| AML ‡ | 2/2 (100) | 0/2 (0) |
| CML** | 0/1 (0) | 0/1 (0) |
| CML, blasts crisis | 4/4 (100) | 0/4 (0) |
| Total leukemias | 17/40 (43 | 0/29 (0) |
| Lymphomas | 22/43 (51) | 2/14 (14) |
| Carcinomas | 0/12 (0) | 0/11 (0) |

* Acute lymphocytic leukemia.
+ Chronic lymphocytie leukemia.
‡ Acute myelogenous leukemia.
** Chronic myelogenous leukemia.

Table 8. Cell-Mediated $^{51}$Cr Release Assay – Autologous Target Cells

| Attacking Lymphocytes | Tests Positive/Total Tests (%+) | |
|---|---|---|
| | Target Cells | |
| | Blasts | Remission |
| Normal | | 0/30 (0) |
| ALL* | 8/20 (40) | 0/22 (0) |
| AML+ | 6/19 (32) | 0/10 (0) |

*Acute lymphocytic leukemia.
+Acute myelogenous leukemia.

The specificity of the antigens detected by lymphocyte stimulation is not completely clear. Some experiments have been performed with remission bone marrow as well as with relapse marrow (10). In some experiments, both blast cells and remission cells produced some stimulation. It remains to be determined whether the stimulation assay is detecting a differentiation antigen present on normal blast cells as well as on leukemic blasts.

In vitro cytotoxicity reactions have been particularly stressed as likely analogues of the cell-mediated defense against tumors (25). Our laboratory has tested the lymphocytes of patients with acute leukemia and of normal individuals against autologous and allogeneic target cells, by a $^{51}$Cr release cytotoxicity assay (10, 26). Table 8 gives a summary of results obtained against autologous target cells. There was no reactivity of normals against their own lymphocytes. Both ALL and AML patients reacted against their blast cells, but not against their remission cells. The observed reactivity did not against their remission cells. The observed reactivity did

not correlate with clinical state. There was at least as much reactivity during bone marrow relapse as there was during remission. The results of tests against allogeneic target cells are given in Table 9. Only a low incidence of reactivity was observed against lymphocytes of normal individuals. In contrast, many positive reactions against blast cells were seen; lymphocytes from leukemia patients and also from normal controls had cytotoxic effects. Positive results were also seen against remission lymphocytes of the leukemia patients.

The cytotoxicity assay appears to be detecting leukemia associated antigens, but the specificity of the reactions may be different from that of the skin tests. In the studies with autologous cells, antigens were only detected on blast cells. In the allogeneic tests, some results with remission cells were also positive. It is likely that the remission cells contain antigens different from those on blast cells, and which are undetectable by skin tests.

There have been few studies of leukemia patients thus far with the leukocyte migration inhibition assay. However, using the assay of Rosenberg et al (27), Halterman et al (28) studied a pair of identical twins, one with leukemia. Leukemic antigen extract, which gave a positive skin reaction in the patient, also caused inhibition of the migration of her leukocytes. The same extract did not affect the migration of the normal twin's leukocytes.

To determine the possible relationships between skin testing, lymphocyte stimulation, and $^{51}Cr$ cytotoxicity assays, the three tests were performed on 20 patients (10). The results are summarized in Table 10. As noted above, results of skin tests correlated with clinical status. The in vitro assays did not correlate with stage of disease, nor did they correlate with each other. The reasons for the lack of correlations are not clear. It is quite possible that each assay is measuring different antigens. Leukemias in experimental animals have been shown to have a complex variety of antigens (29, 30). Studies with isolated, soluble antigens are now feasible and should help to decide this issue. The assays may also be measuring different phases of the immune response, and different subpopulations of lymphocytes may be responsible for the various effects. It is quite possible that the lymphocyte stimulation assay measures the primary recognition of foreign cell surface antigens.

**Table 9. Cell-Mediated $^{51}Cr$ Release Assay – Allogeneic Target Cells**

| Target Cells | Tests Positive/Total Tests (%+) | | |
|---|---|---|---|
| | Attacking Lymphocytes | | |
| | Normal | ALL Patients | AML Patients |
| Normal lymphocytes | 5/220 (2.3) | 0/20 (0) | 0/15 (0) |
| ALL* blasts | 61/134 (46) | 10/24 (42) | 4/15 (27) |
| ALL remission cells | 20/93 (22) | 2/16 (13) | 1/7 (14) |
| AML+ blasts | 41/100 (41) | 6/15 (40) | 6/25 (24) |
| AML remission cells | 15/50 (30) | 0/4 (0) | 0/5 (0) |

*Acute lymphocytic leukemia.
+Acute myelogenous leukemia.

Table 10. Results of three assays of cellular immune reactivity in acute leukemia to autochthonous blasts cells

| Patients | Tests positive/total number of tests | | |
|---|---|---|---|
| | Skin Tests | Lymphocyte Cytotoxocity | Mixed Leukocyte Culture |
| ALL, relapse | 1/3 | 1/2 | 2/3 |
| ALL, remission | 6/9 | 4/10 | 6/9 |
| AML, relapse | 2/5 | 2/3 | 3/6 |
| AML, remission | 8/9 | 2/10 | 9/14 |
| Total | 17/26 (65 %) | 9/25 (36 %) | 20/32 (63 %) |

The lymphocyte cytotoxicity assays appear to detect the presence of presensitized cells, capable of rapidly reacting with the target cells. The skin tests are thought to depend on sensitized lymphocytes capable of releasing migration inhibitory factor and other soluble mediators, and also on the presence of adequate numbers of mononuclear cells to accumulate at the reaction site.

### Use of Immunological Assays to Monitor Immunotherapy

There has been much recent interest in the use of immunotherapy in acute leukemia. Most of the studies already performed have been empirical, without assessment of the antigenicity of the immunizing cells and without immunological monitoring of the immune response to the therapy.

An immunotherapy study was performed on previously treated ALL patients, in which allogeneic ALL blast cells plus either BCG or methotrexate were given (24). Immune responses were serially determined, by skin testing and by lymphocyte stimulation. The most dramatic change was in the response of the BCG treated group to PPD. Skin tests with allogeneic blast extracts did not provide clear evidence for immunization, even against the HL-A antigens of the donor cells. There was, however, a correlation between skin reactivity against the extract of donor cells and the duration of remission. In the lymphocyte stimulation assays, there was also little evidence for immunization by the donor cells.

The use of these immunological assays could help in designing future immunotherapy trials. Allogeneic cells could be selected on the basis of their ability to elicit skin reactions, thereby documenting that the donor cells possess common antigens. In addition, monitoring with skin tests could be used to determine the immunogenicity of different immunizing preparations and schedules. It would be very helpful if autologous cells were available for skin testing and for in vitro tests, since they would permit a distinction between immunization to leukemia associated antigens and immunization to normal histocompatibility antigens. If an immunotherapy trial were clinically successful, it would provide important information on the predictive value of each of the assays.

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