Immune Function at Diagnosis in Relation to Responses to Therapy in Acute Lymphocytic Leukemia of Childhood

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Tests of immune capacity were performed on blood from 49 children with newly diagnosed, untreated acute lymphocytic leukemia, and relation to prognosis was determined. Patients were treated with multiple-drug therapy and prophylactic cranial irradiation. Median follow-up time was 16 mo (range 10–37 mo). Principal unfavorable findings at diagnosis were absolute numbers of T lymphoid cells outside the range 850–2500/μl blood, absence of whole blood responses to phytohemagglutinin in vitro, a low titer of complexed antibody, and the presence in serum of free leukemic blast cell membrane antigen. Fourteen patients showed two or more unfavorable findings at diagnosis. Eleven of these have died. Four of the remaining 35 patients have died. A shorter duration of first remission was found among patients with abnormal numbers of T cells at diagnosis. The findings suggest that the immunologic capacity of the patient at diagnosis is an important determinant in responses to therapy.

A S S E S S M E N T of the immune capacity of patients with acute leukemia at diagnosis has been technically difficult, particularly in the presence of peripheral blood blast cells, and has led to conflicting results. Antibodies to leukemia antigens were detected by Doré et al.1 in only 12 of 51 patients using four different techniques. Others have demonstrated antibodies only in remission.2,3 Cellular immunity to leukemic cells has not been demonstrable in patients with blastemia, either by skin test with leukemia antigens, or by in vitro assays, but autologous cellular immunity has become evident in patients in remission by skin testing for delayed sensitivity, in vitro cytotoxicity, blastogenesis, or antibody-directed cell-mediated immunity using autologous leukemic blasts.4–11 Gutterman et al.12 reported that serum from 8 of 19 newly diagnosed patients with acute myeloid leukemia and 1 of 5 patients with acute lymphocytic leukemia (ALL) blocked autologous blastogenesis to leukemic antigens, and the presence of blocking was correlated with a good prognosis.

There is an obvious need to extend the immunologic assessment of patients with acute leukemia at diagnosis and to correlate results of these investigations with the patient’s response to treatment. We report results of analysis of lymphoid cell populations and soluble immune complexes in the blood of 49 children with untreated ALL at diagnosis. The results of these immunologic investigations have been correlated with the duration of first remission and
survival among the patients treated with multiple-drug chemotherapy and prophylactic cranial irradiation.

MATERIALS AND METHODS

Patients

Blood was obtained at initial diagnosis from 49 children with untreated ALL admitted over a 2-yr period. These patients were not all consecutive admissions and may not reflect a true prevalence of different subgroups of ALL. Patients were treated with induction chemotherapy, most commonly vincristine and prednisolone, followed by prophylactic cranial irradiation (2400 rads) and four intrathecal methotrexate injections. Continuation therapy with methotrexate, 6-mercaptopurine, and cyclophosphamide was given after remission induction. Blood was taken for remission antibody between 3 and 6 mo after remission induction. Duration of first remission was calculated until bone marrow relapse occurred.

Lymphoid Cell Subpopulations

Mononuclear leukocytes were purified from heparinized blood at initial diagnosis by discontinuous Ficoll-Hypaque density gradient centrifugation (S.G. 1.077, 400 g, 20 min, 22°C). This population of cells, upon which studies of three membrane markers were performed, contained mature lymphocytes, monocytes, and leukemic blast cells. Membrane immunoglobulin (MIg) was detected using fluoresceinated goat anti-human immunoglobulin sera (Hyland Division, Travenol Laboratories, Inc., Costa Mesa, Calif.). C3 membrane receptors were detected using the EAC rosette method. As whole rabbit anti-sheep red cell serum was used, any rosettes formed could have included Fc receptor-bearing cells (EA rosettes). T-cell rosettes (“cold” E rosettes) were formed by incubating sheep red cell patient lymphoid cell mixtures at 37°C for 15 min followed by 2-4 hr at 4°C before reading. Primitive E rosettes (“hot” E rosettes) were formed by reading after incubation of cell mixtures at 37°C for 1-2 hr. Absolute numbers of cells/μl blood were calculated by total leukocytes/μl x (% lymphocytes + % monocytes x % rosettes. Using similar techniques, 32 normal children ranging in age from 1 to 11 yr tested in our laboratory have had a mean and SD of 1667 ± 410 T cells, 986 ± 270 C3 cells, and 562 ± 194 MIg cells/μl blood. A normal range of 850-2500 T cells was calculated using the mean ± 2 SD.

Leukemia Cells

Cells from bone marrow aspirations before start of therapy were freed from granulocytes, red cells, and platelets by Ficoll-Hypaque density gradient centrifugation and washing. Aliquots of separated lymphoblasts were quick-frozen and stored in liquid nitrogen. Other aliquots were directly labeled with 125I for assay of membrane antigens.

Radio-iodination of Cell Membrane

Surface-membrane proteins of separated lymphoblasts were directly labeled with carrier-free 125I (Radiochemical Centre, Amersham, England) by the lactoperoxidase method of Marchalonis et al. In this method, 2 x 10⁷ lymphoblasts were suspended in 50 μl phosphate-buffered saline, pH 7.2 (PBS), and 50 μl lactoperoxidase (Calbiochem, San Diego, Calif.), 5 μl 125I (200 mCi/ml), and 10 μl 0.03% hydrogen peroxide were added. Addition of lactoperoxidase and H2O2 were repeated twice at 5-min intervals. Cells were then washed three times in cold PBS and finally suspended in 1 ml RPMI 1640 tissue culture media (Commonwealth Serum Laboratories, Melbourne) without serum and incubated for 2 hr at 37°C. The supernatants contained 90% of 125I-labeled protein from lymphoblast membranes shed by spontaneous metabolic turnover. These were dialyzed with 0.01 M PBS for 48 hr at 4°C and lyophilized. This preparation was used as leukemia cell membrane turnover antigen (MTO Ag).

Radioimmuno-Counter Electrophoresis

Relative specific immune binding between 125I-MTO Ag and specific antibody in autologous serum taken in remission was detected by radioimmuno-counter electrophoresis as previously described. Optimum MTO Ag concentrations were determined for each patient by preliminary
titration against autologous remission serum and control serum at 1:100 dilution in low ionic strength buffer of 0.01 M Tris, 0.1 M NaCl, and 0.001 M ethylenediaminetetraacetic acid (EDTA) at pH 9.0 (ionic strength 0.2). This optimum concentration of MTO Ag was used to detect free antibody in autologous serum taken at diagnosis (low-salt buffer) or bound antibody released from immune complexes in sera by high ionic strength buffer of 0.01 M Tris, 3 M NaCl, 0.001 M EDTA, pH 9.0 (ionic strength 1.6), and electrophoresis. Results were expressed as the titer of serum at which maximum binding of $^{125}$I-MTO Ag occurred relative to a normal control serum.

Free or bound leukemia antigen was detected in sera taken at diagnosis by measuring specific binding by $^{125}$I-labeled gamma globulin prepared from autologous remission sera as previously described. Sera were assayed in low ionic strength buffer for detection of free antigen and in high ionic strength buffer for detection of antigen bound as an immune complex. Results were expressed as the titer of remission gamma globulin at which maximum binding occurred relative to a normal control serum. A low titer of maximum binding occurred when a large amount of free antigen was present. Control MTO Ag was also prepared from bone marrow cells of six patients after remission induction and relative specific binding tested with autologous sera. Allogeneic specificity of these reagents will be reported elsewhere.

Responses to Phytohemagglutinin

The level of spontaneous incorporation of tritiated thymidine into DNA of blood leukocytes exposed to optimum concentrations of phytohemagglutinin (PHA) was measured by a whole blood method. Fifty microliters of heparinized whole blood from patients at initial diagnosis was diluted in 100 µl RPMI 1640 tissue culture medium containing 0, 50, 200, or 500 µg PHA-M (Wellcome Laboratories, Beckenham, England). Cultures were incubated for 24 hr at 37°C, pulsed with 5 µCi tritiated thymidine (Radiochemical Centre) and terminated at 48 hr. Red cells were removed by washing with water on a membrane filter. Results were expressed as counts per minute of tritium in trichloroacetic acid-precipitated material per unit blood volume. Statistical significance of differences in lymphoid cell numbers among patients in different groups was determined by the Wilcoxon Mann Whitney Rank Sum analysis. Statistical analysis of actuarial remission rates was performed by a rank-order method.

RESULTS

Patients

The 49 children studied ranged in age from 2 mo to 13 yr-9 mo (median age 4 yr-9 mo) and consisted of 27 boys and 22 girls. Of these, 48 achieved bone marrow remission and 22 remained in first complete remission with a median follow-up time of 16 mo (range 10–37 mo); 26 have relapsed (median time to relapse, 10 mo, range 2–29 mo) and 14 of these have died. One patient, aged 2 mo, who failed to achieve bone marrow remission had total blood leukocyte and T-cell numbers at diagnosis of 57,000 and 10,300, respectively.

Total leukocyte numbers and mature lymphoid cell subpopulations/µl blood at diagnosis are shown in Table 1 for all 49 patients. The proportions of T cells in patients with leukocytosis > 30,000/µl ranged from 0.2% to 68% (median 6%); in those with 10,000–30,000 leukocytes/µl the range was 0.6%–36% (median 10%); and in those with < 10,000 leukocytes/µl the range was 7%–67% (median 28%) per total mononuclears. Both small lymphocytes and large lymphoblasts formed E rosettes, but populations of leukemic or normal T-cell reactive blasts could not be defined. Significant ($p < 0.05$) increases in T-cell numbers were found in patients with leukocytosis of over 30,000/µl blood when compared to T-cell numbers in normal children or in patients with total leukocytes less than 30,000 at diagnosis. Equivalent increases in $C_3$ receptor-bearing cells or $M_{1g}$-bearing cells were not seen in patients with over 30,000 leukocytes. Reduced
Table 1. Blood Lymphoid Cell Subpopulations According to Total Leukocyte Counts in Patients at Diagnosis

<table>
<thead>
<tr>
<th>Total Leukocytes/μl Blood</th>
<th>No. of Patients</th>
<th>Lymphoid Cell Populations (Median and Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T</td>
</tr>
<tr>
<td>&gt;30,000</td>
<td>13</td>
<td>a. 3217</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10,000–30,000</td>
<td>13</td>
<td>b. 1500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10,000</td>
<td>23</td>
<td>c. 1278</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T, E-rosette-forming cells; C3, EAC-rosette-forming cells; Mlg, lymphoid cells with membrane-bound immunoglobulin.

a:b, p < 0.01. b:c, N.S. a:c, p < 0.01.

Numbers of C3- and Mlg-bearing cells were found in patients with less than 10,000 leukocytes/μl blood but showed no relationship with prognosis.

T-Cell Number and Prognosis

Patients were arranged into three groups according to the absolute T-cell numbers/μl blood at diagnosis: 26 patients had initial numbers of T cells within the range 850–2500/μl blood; 16 had more T cells (median 3873, range 2592–34272/μl); and 7 had fewer T cells (median 600, range 412–830/μl).

Mortality was significantly higher (p < 0.05) in patients with increased numbers of T cells, compared to patients whose T-cell numbers were in the normal range (Table 2). Four children in the high T-cell number group had over 20% of lymphoblasts forming E rosettes stable at 37°C (“hot” rosettes). One of these died without achieving remission, two have relapsed and died subsequently, and the remaining child is in first remission of 9 mo duration. None showed evidence of mediastinal lymphoma. The remaining patients with increased numbers of cold E-rosette-forming cells showed less than 1% of “hot” E rosettes in blood and differentiation could not be made between normal and leukemic T lymphoblasts.

An increased mortality was also seen in seven patients with low numbers of T cells at diagnosis, but numbers in this group were too small for this trend to be significant (0.3 > p > 0.2). Of these seven children, three of four with pe-

Table 2. T-Cell Number and Prognosis

<table>
<thead>
<tr>
<th>Total T-Cell No.</th>
<th>Total No. of Children</th>
<th>In First Remission (Median Follow-up, mo.)</th>
<th>Relapsed (Median Time, mo.)</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;2500</td>
<td>16</td>
<td>4 (14)</td>
<td>12 (9)</td>
<td>a. 8</td>
</tr>
<tr>
<td>2500–850</td>
<td>26</td>
<td>13 (16)</td>
<td>13 (9)</td>
<td>b. 4</td>
</tr>
<tr>
<td>&lt;850</td>
<td>7</td>
<td>4 (13,15,17,21)</td>
<td>3 (4,6,9)</td>
<td>c. 3</td>
</tr>
</tbody>
</table>

a:b, p < 0.05; b:c, 0.3 > p > 0.2.
Fig. 1. Actuarial analysis of duration of first continuous remission in patients grouped according to total blood T-cell number at diagnosis. P, proportion of patients in first remission; mo, months of observation.

Peripheral blood leukemic blasts at diagnosis have died, while three patients with no peripheral blood lymphoblasts at diagnosis remain in first remission of 1–21 mo duration.

Actuarial first remission rates for patients with increased, normal, or decreased numbers of T cells at diagnosis are seen in Fig. 1. Differences between patients with increased or normal numbers of T cells become significant at 14 mo (p < 0.1).

Response to PHA

Forty-five patients were tested for whole blood blastogenic responses to PHA (Fig. 2). Six patients with no peripheral blood blast cells at diagnosis had normal background levels of spontaneous tritiated thymidine incorporation, and four showed increases in thymidine uptake of normal magnitude in cultures with PHA. All these patients remain in first remission.

Increased spontaneous incorporation of tritiated thymidine was found in all patients with peripheral blood lymphoblasts. In 37 patients an increase in triti-
ated thymidine incorporation was found with one or more concentrations of PHA, suggesting a response by a subpopulation of normal T cells. Seven of these patients have died. Eight children, all of whom have died, showed increased spontaneous thymidine uptake and either no change or a decrease in thymidine uptake with PHA. Of these, five had over 3500 T cells/µl blood at diagnosis, and three had less than 800 T cells/µl blood at diagnosis.

Antibody to ALL-Blast Membrane

125I-labeled membrane turnover protein from autologous (14 children) or allogeneic (16 children) bone marrow leukemic blasts was used to detect specific antibody in serum taken at initial diagnosis (Fig. 3A). No binding of 125I-labeled antigen could be detected during electrophoresis of initial sera from any patient when diluted in low ionic strength buffer, suggesting the absence of free specific antibody. Binding at serum titers ranging from 1/40 to 1/5000 occurred when electrophoresis was performed upon sera in high ionic strength buffer, suggesting that antibody is released from an immune complex. Bound antibody at titers of 1/160 or less was found in sera from seven patients. All of these patients had more than 2500 T cells/µl blood at diagnosis, and all have died. No relationship was found between the titer of bound antibody greater than 1/160 and either T-cell number or prognosis. No differences were found between titers with autologous or allogeneic antigen. Control antigens were prepared from bone marrow cells of six patients in remission whose initial serum had been previously tested with autologous antigen from leukemic marrow. No binding

Fig. 3. (A) Titer of serum taken at diagnosis at which maximum binding with autologous MTO Ag occurred during counter electrophoresis. No binding found in sera run in low ionic strength buffer. Median and range of titers of binding in sera run in high ionic strength buffer. A, patients with < 2500 T cells/µl; B, patients with > 2500 T cells/µl. (B) Titer of 125I-gamma globulin from autologous remission serum at which maximal binding with antigen in initial sera occurred. Sera from eight patients showed binding during counter electrophoresis in low ionic strength buffer. Slight increases in amounts of antigen detected were found when run in high ionic strength buffer (shown individually). C, median and range of antigen in 16 sera run in low and high ionic strength buffer showing significant increase in amount of antigen released.
Table 3. Determination of Titer of Free and/or Bound Antigen in Patients' Initial Serum Using Radioimmuno-Counter Electrophoresis

<table>
<thead>
<tr>
<th>Titer of Patient Remission $^{125}$I-Gamma Globulin</th>
<th>Low-Salt (0.2 M) Buffer</th>
<th>High-Salt (1.6 M) Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient*</td>
<td>Control*</td>
</tr>
<tr>
<td>Serum A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3037/8326</td>
<td>2986/8231</td>
</tr>
<tr>
<td>5</td>
<td>3399/7941</td>
<td>3066/8159</td>
</tr>
<tr>
<td>10</td>
<td>3215/8211</td>
<td>3158/8311</td>
</tr>
<tr>
<td>20</td>
<td>3364/8332</td>
<td>3003/8301</td>
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<td>50</td>
<td>4103/8215</td>
<td>3289/8298</td>
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<td>100</td>
<td>5943/7883</td>
<td>3162/8186</td>
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<tr>
<td>250</td>
<td>3311/8064</td>
<td>3044/8177</td>
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<tr>
<td>500</td>
<td>3215/8091</td>
<td>3161/8205</td>
</tr>
<tr>
<td>1000</td>
<td>4131/8156</td>
<td>3109/8173</td>
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<tr>
<td>2000</td>
<td>3091/8039</td>
<td>3118/8319</td>
</tr>
<tr>
<td>Serum B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2876/6934</td>
<td>2635/6842</td>
</tr>
<tr>
<td>5</td>
<td>2934/6986</td>
<td>2682/6936</td>
</tr>
<tr>
<td>10</td>
<td>2883/6918</td>
<td>2719/6915</td>
</tr>
<tr>
<td>20</td>
<td>2896/6937</td>
<td>2786/6922</td>
</tr>
<tr>
<td>50</td>
<td>2936/6993</td>
<td>2764/6892</td>
</tr>
<tr>
<td>100</td>
<td>2711/7081</td>
<td>2698/6954</td>
</tr>
<tr>
<td>250</td>
<td>2834/6949</td>
<td>2736/6932</td>
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<tr>
<td>500</td>
<td>2891/6924</td>
<td>2788/6942</td>
</tr>
<tr>
<td>1000</td>
<td>2801/7009</td>
<td>2732/6913</td>
</tr>
<tr>
<td>2000</td>
<td>2734/6983</td>
<td>2784/6891</td>
</tr>
</tbody>
</table>

*Initial patient or normal control serum diluted with low or high ionic strength buffer. Counts per minute in reaction zone/cpm at origin of electrophoresis slide = $r$.

1 $R = r_{patient}/r_{control} = 1.00$ when no specific binding of $^{125}$I remission gamma globulin occurs. Peak values of $R$ indicate free antigen at 1/100 and total antigen at 1/20 (serum A). Serum B shows no free antigen in low ionic strength buffer but antigen present at 1/10 in high ionic strength buffer.

Antigen in Sera

Remission serum was taken between 4 and 8 mo after initial diagnosis in 24 patients and the $^{125}$I-labeled gamma globulin was used to detect antigen in autologous sera taken at initial diagnosis (Fig. 3B). Binding of $^{125}$I-gamma globulin occurred in initial sera of eight patients when run in low ionic strength buffer, suggesting the presence of free antigen (Table 3, serum A). All sera tested in high ionic strength buffer similarly showed binding, but only small increases in binding occurred in sera with free antigen. Dilution in high ionic strength buffer of the 16 sera showing no free antigen resulted in much greater degrees of binding during electrophoresis (Fig. 3B, Table 3, serum B). These data were interpreted as suggesting the presence of soluble antigen in excess in eight sera, with a small amount of additional antigen bound by antibody and released in high-salt buffer. The majority of sera showed no free soluble antigen but relatively large amounts of antigen bound by antibody which was released by high-salt buffer and electrophoresis.
Seven of the eight patients showing free antigen in their serum at diagnosis have died and six had more than 2500 T cells/μl. Total leukocyte counts in these children ranged from 2500 to 240,000/μl blood (median 57,600/μl). One of these patients who died without achieving remission had his initial serum tested with allogeneic remission serum, which showed the presence of free antigen. Of the 16 children who showed only bound antigen in initial sera, four have died, five have relapsed but are still alive, and seven remain in first remission. No clear relationship between amount of bound antigen detected and the prognosis was found. Total leukocyte counts in blood from these 16 children ranged from 3300 to 270,000/μl (median 11,700/μl).

**Prognosis With Multiple Unfavorable Tests**

An abnormal number of T cells, failure to show an increment in whole blood tritiated thymidine uptake with PHA, a serum titer of bound antibody to leukemic blast cell membrane of 1/160 or less, and the presence of free serum antigen in blood at diagnosis were each considered unfavorable findings: 6 children showed all four unfavorable findings, and 5 of these are dead; 6 children showed two unfavorable findings, and 5 of these are dead; 13 children showed one unfavorable finding (usually T cells > 2500), and 2 died; 22 children showed no unfavorable findings, and 2 died. Thus, 14 patients showing two or more unfavorable findings accounted for 11 deaths, whereas only 4 deaths have occurred among the remaining 35 patients. Initial median blood leukocyte counts were 57,600/μl (range 270,000–6,800) in the 14 patients with two or more unfavorable tests, and 8,700 (range 65,000–1,000) in the remaining 35 patients. There were 8 males in the group with two unfavorable tests, and 22 males among the remaining 35 patients.

**DISCUSSION**

Multiple-drug chemotherapy and the prophylactic treatment of leukemic cells in the central nervous system have resulted in an increasing proportion of long-term survivors in childhood ALL. The findings in this study suggest that the immunologic responses of the host may be an important determinant in the response to treatment. Abnormalities of thymus-derived lymphoid populations, or the presence of a gross excess of “systemic leukemia antigen” beyond the neutralizing capacity of the host were associated with a poor response to treatment. The absolute number of T lymphocytes ("cold" E rosettes) at diagnosis was between 850 and 2500/μl in over 50% of the patients, and these showed the most favorable response to therapy (Table 2 and Fig. 1).

Patients with numbers of T cells greater than 2500/μl of blood at diagnosis responded poorly to therapy, with 12 of 16 relapsing early during continuation chemotherapy. Four of these children also showed abnormal T cells (“hot” E rosettes) which had characteristics of normal thymus or malignant lymphoma cells. The numbers of abnormal T cells in the blood of these children suggest a differentiation from the malignant clone, rather than an excessive host reaction with primitive thymic cells appearing in the blood. The origin of increased numbers of cells with T-cell characteristics in four additional patients with gross excess of soluble leukemic antigen in blood and poor prognosis was not clear. Previous studies have reported up to 20% of patients with ALL to have high
proportions of lymphoblasts with T-cell characteristics. A number of these patients also had an associated mediastinal lymphoma, and their reported prognosis has been poor.31 These cases may represent a disease subgroup in which some differentiation of leukemic cells along the T-cell pathway has occurred.15 Of seven patients with T-lymphocyte numbers less than 850/μl at diagnosis, those children with leukemic blastemia responded poorly to therapy, while a good response was found where no peripheral blood lymphoblasts were present, although numbers in this group were too small for adequate statistical evaluation.

Antigens on human leukemic cells which are recognized by autologous human patients have not been easy to demonstrate. The reported variation in specificity, sensitivity of detection, and localization of antigens within the cell32,33 suggests that several antigens may be present and that antisera raised by inoculation of human tumor cells into subprimate species may recognize different antigens from autologous human or primate antisera.34,35 Our results with human antisera and labeled autologous leukemia membrane suggest that all of the 49 children studied at diagnosis had antibody in the blood bound to a soluble serum antigen with similar specificity as soluble membrane turnover antigen derived from autologous leukemia cells in vitro. In six patients tested, a similar antigen could not be demonstrated in membrane turnover protein from normal autologous bone marrow cells taken in remission, nor could antigen of similar specificity be detected in membrane-derived protein from four other types of childhood malignant tumors.

Allogeneic specificity of these human reagents, to be reported elsewhere in detail,20 is summarized as follows. Thirteen of 43 sera from patients with ALL in remission bound only membrane preparations from lymphoblastic leukemic cells (16 patients), but not from other forms of acute leukemia, malignant lymphoma, solid malignant tumors, normal bone marrow, or other tissues. Twenty-five of these 43 sera bound membrane preparations of blast cells from several types of leukemia and malignant lymphoma, but not from other malignant tumors, normal thymus, or normal bone marrow. Five of the 43 sera reacted widely with both malignant and normal tissues of several individuals, but not with autologous remission bone marrow cells. The fully autologous human system may be the most reliable measure of leukemia-associated immunity, but these data suggest that many (88%) allogeneic remission sera may detect membrane antigens associated with lymphoid cell malignancy which are widely cross-reactive. The disease specificity of these reactions makes nonspecific reactions, as to Fc receptors on leukemic cells or anti-HL-A activity, unlikely in the majority of sera.

The quality of the patients' antibody response in terms of total capacity to bind soluble leukemic blast cell membrane-derived antigens (Fig. 3B) appeared to correlate with duration of first remission and with survival. High avidity of binding of these soluble complexes has been previously found in long-term survivors of ALL.36 Similar soluble immune complexes in blood of children with neuroblastoma and Wilms' tumor have been suggested to play a functional role in blocking cellular antitumor immunity in those patients.17 The administration of BCG vaccine in combination with intermittent chemotherapy to children with ALL in remission has been observed to increase the titer of anti-
body to autologous and allogeneic leukemic blast cell membrane-derived antigen, but its influence upon avidity has not yet been determined.

Neutralization of antileukemia antibody by soluble antigen in blood of newly diagnosed patients may account for previous difficulties in detecting antibody in untreated patients with this disease. Circulating immune complexes could also account for dermal anergy to leukemia antigens and serum blocking activity in leukemia sera at diagnosis. Although serum blocking activity for autologous blastogenesis was not tested in this study, previous work demonstrated maximal blocking in sera containing immune complexes of tumor membrane antigens at or near equivalence, with diminution in blocking in the presence of antigen excess. In this study, patients with immune complexes in serum and no detectable antigen excess responded well to therapy, an observation which may account for the better prognosis of leukemic patients found to have blocking activity in serum.

Incorporation of tritiated thymidine into unstimulated cultures of whole blood was related to the numbers of leukemic blasts, and, in some patients, was 500-fold higher than in normal children (Fig. 2). In spite of these high counts in unstimulated cultures, the majority of patients showed a further increment in thymidine incorporation at one or more concentrations of PHA, suggesting a response by a small subpopulation of T cells. Eight patients who failed to show this increment with PHA have died (Fig. 2).

An analysis of the immunologic function studies of children who have died or relapsed in comparison with those in remission showed that 11 of 14 children who had two or more abnormalities of immunologic function died. In contrast, only 4 of 35 with one or no immunologic abnormalities died. Our results clearly indicate that children with poor response to antileukemic treatment have profound immunologic abnormalities. It would seem rational to employ means of boosting the immune response as a means of improving the prognosis of these children. Such clinical studies are underway in our institution.

REFERENCES

20. Jose DG: Manuscript in preparation
Immune function at diagnosis in relation to responses to therapy in acute lymphocytic leukemia of childhood

DG Jose, H Ekert, J Colebatch, K Waters, F Wilson and D O'Keefe