Interleukin 2 Induction of Lymphokine-Activated Killer (LAK) Activity in the Peripheral Blood and Bone Marrow of Acute Leukemia Patients. I. Feasibility of LAK Generation in Adult Patients With Active Disease and in Remission

By Aliza Adler, Paul A. Chervenick, Theresa L. Whiteside, Eva Lotzová, and Ronald B. Herberman

The feasibility of in vitro interleukin 2 (IL-2) activation and expansion of mononuclear cells (MNCs) derived from adult patients with acute myelogenous leukemia (ANLL) was studied. Patients' natural killer (NK) and lymphokine-activated killer (LAK) cell activity was compared with that of normal donors in terms of: (a) cytolytic activity (four-hour 51Cr release assay) against an NK-sensitive target (K562), NK-resistant targets (Raji/Daudi), and fresh/cryopreserved autologous and allogeneic leukemic blasts; (b) proliferation and expansion in culture with 1,000 U/mL recombinant IL-2 (rIL-2); and (c) the cell surface phenotype of the cultured cells. In 21 of 24 patients with active disease (AP) MNCs derived from the peripheral blood (PBL) or bone marrow (BM) could be cultured and expanded in the presence of rIL-2. These cultures initially contained between 30% and 50% blasts, and during 2 to 4 weeks of culture destruction of blasts and enrichment of up to 60% in cells with the morphology of large granular lymphocytes (LGLs) was observed. Expansion in culture varied between 2- and 100-fold. MNCs from all patients in remission (RP) could be activated by rIL-2 and expanded up to 30-fold after 1 to 3 weeks in culture. NK activity of fresh PBLs from AP was significantly lower than in normal controls, whereas NK activity of RP was within the normal range. High levels of postactivation NK and LAK activity on K562/Raji/Daudi and on fresh/cryopreserved leukemic blasts was generated in ~50% of cases of AP and in most RP. Cell surface phenotype studies showed that cultured cells derived from ANLL patients were significantly enriched (up to 40%) in NKH-1 (Leu 19) positive cells, with RP LAK cells also expressing a high proportion of CD16 positive cells (up to 40%). This study has shown that it is feasible to activate and significantly expand killer cells derived from active disease and remission ANLL patients during 1 to 3 weeks culture with IL-2 with good maintenance of cytolytic activity. Both initial NK activity and LAK generation was optimal in remission patients. Based on data from this study, a clinical protocol has been developed for treatment of early relapse ANLL patients with LAK cells cultured for 1 to 3 weeks and systemic IL-2.

The main objective of the present study was to determine suitable conditions for ex vivo activation and expansion of lymphoid cells derived from adult, acute leukemia patients as a basis for the design of clinical therapy protocols.

Two groups of patients were studied: (a) ANLL patients at the time of diagnosis or relapse were designated active patients (APs), and (b) ANLL patients in remission were designated remission patients (RPs).

No reports exist of studies of the generation of LAK activity in RPs, and this information seemed particularly important to gather since cells obtained from RP might provide an excellent source for treatment of their subsequent relapse.

MATERIALS AND METHODS

Patientmaterial. Forty-one samples of PBL and/or bone marrow (BM) were collected from acute leukemia patients during this study. Of these, 24 were from patients with active disease (AP) and 11 from patients in remission (RP). Two patients were tested both during active phase of disease and again upon remission. BM samples were received from 17 APs and in 16 cases enough blasts were recovered and cryopreserved for use as targets in subsequent in vitro studies.

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cytotoxicity tests. A summary of the patients studied is presented in Table 1.

PB and/or BM-derived lymphocytes were cultured in rIL 2 (Cetus, Emeryville, CA), and their antitumor potential was assessed by cytolytic activity against NK-sensitive and NK-resistant targets and against noncultured, autologous, or allogeneic leukemia blasts. The NK and LAK activity of PB or BM lymphocytes of APs and RPs was compared with the cytolytic activity of lymphocytes from normal individuals, designated as normal (N) PBL or N LAK. This study was approved by the Institutional Review Board, and all donors of PBL or BM signed the informed consent form.

**Cell separation techniques.** PB or BM was collected in preservation-free heparin. PBL from N and RPs was separated by the standard Ficoll-Hypaque procedure (Pharmacia, Piscataway, NJ). PBL or BML from APs was separated by two layer Ficoll-Hypaque: 100% and 75%. The top layer, enriched for leukemia blasts, was used for targets (fresh or cryopreserved); the bottom layer enriched for MNCs was cultured in rIL 2. Blasts were cryopreserved in a controlled linear rate freezing apparatus (Cryomed, Ml Clemens, MI) in 90% human AB serum and 10% DMSO. Leukemic blast preparations used as targets for cytotoxicity assays contained ~90% blasts, as determined by cyto- spin smears.

**Culture with rIL 2.** MNCs were suspended at 10^6/mL in culture medium containing 1,000 U/mL of rIL 2 (generously provided by Cetus) and cultured in 24-well plates (Costar) or flasks (Falcon) in upright position. The culture medium used was RPMI supplemented with 2% heat-inactivated human AB serum (MSI, Boston), with 2 mmol/L L-glutamine and penicillin (100 U/mL)—streptomycin (100 µg/mL) mixture (GIBCO, Grand Island, NY). Cells were counted every 2 to 4 days and re-fed with fresh medium and IL 2, and the cell concentration was readjusted to 10^6/mL.

Cultures were kept in 5% CO2 at 37°C. An expansion index (EI) was calculated for each culture (fold expansion/culture).

Cytospin preparations (Cytospin-2, Shandon, Sewickley, PA) were done before culture and at 1, 2, and 3 weeks in culture and were stained by May-Grunwald-Giemsa. The preparations were examined in a light microscope for the presence of blasts and of LGLs. The percentage of blasts in the initial cultures from APs varied from 30% to 50%.

**Cytolytic activity.** The standard four-hour 51Cr release assay was used. For K562, Raji and Daudi 100 µCi of 51Cr was used for one-hour labeling, whereas fresh leukemia blasts were labeled with 200 to 250 µCi for two hours. The spontaneous release was 10% to 25%, and never exceeded 30%. Effector to target (E:T) cell ratios of 50:1, 25:1, 12.5:1, and 6.25:1 were used routinely. Lytic units (LU) were calculated at the 20% level for 10^6 effectors. Total lytic units (TLU)/culture were calculated as Ei x LU.

**Leukemia blast cell targets in cytotoxicity assays.** The cryopreserved blasts were thawed rapidly in a 37°C water bath and resuspended slowly in RPMI medium. Cells were washed twice and counted in trypan blue. Cytotoxicity experiments were performed only with blast preparations of at least 80% viability.

The neuraminidase (NA) treatment of cryopreserved-thawed blast cells was performed before Cr labeling. NA (Boehringer Mannheim) was used at a concentration of .02 U/2 to 5 x 10^6 blast cells. The mixture was incubated for 30 minutes at 37°C, and the cells were washed three times and used as targets after being labeled with 51Cr.

**Cell phenotype.** Cell surface markers were determined by direct and indirect staining of cell suspensions with monoclonal antibodies (Becton Dickinson Moutain View, CA) directed against: T3 (CD3), T4 (CD4), T8 (CD8), CD16 (Leu 11a), NKH-1 (Leu 19), CD25 (IL 2 R), HLA-DR and analyzed in the FACStar Becton Dickinson 488 nm, five watt, argon laser by one- or two-color analysis.

**Statistical analysis.** Student's t test was used to determine the significance of differences of individual assays, and paired t test was used for significance of differences between two groups.

**RESULTS**

**Cultures of PBL and BML from acute leukemia patients.** PBL from APs contained leukemic blasts in proportions varying between 30% and 80%. In these cases, attempts were made to enrich for MNCs by double-layer Ficoll-Hypaque so that the cell suspensions contained 20% to 50% blasts and 50% to 80% MNCs before culture in rIL 2. These enriched populations were cultured with rIL 2 for up to 4 weeks and tested, whenever enough cells were available, during the first, second, and third weeks in culture.

In 21 of 24 samples from APs, lymphoid cells could be cultured in IL 2 and tested for all study parameters. In ten of 17 samples of BM obtained from APs, enough MNCs were present to attempt culture in IL 2 and in eight of ten these could be expanded and tested. All 12 samples of PB from RPs could be cultured and tested. Normal donors' PBL was run in parallel with patients' cells whenever possible.

In all experiments, cells from leukemia patients were cultured in 24-well plates and whenever the initial total number of cells was at least 5 x 10^6, bulk cultures were done in flasks to simulate conditions for massive LAK cultures for therapy. Both expansion and cytolytic activity were similar when cells were grown in plates or in flasks in upright position at 5 x 10^6 to 10^7 cells/mL. When cells were grown in flasks in the horizontal position, but otherwise under the same conditions, cell yield and cytolytic activity were lower (results not shown). Cultures started in 24-well Costar plates were harvested and transferred to flasks, usually during the second week or whenever at least 2 x 10^7 cells were available.

During the first week in culture, in 11 of 21 cultures from APs, leukemia blasts were destroyed, as monitored by cyto- spin preparations. In the remaining ten cultures, few blasts were detected after 1 week; they subsequently disappeared during the second week in culture with rIL 2 (results not shown).

**Expansion in culture with rIL 2.** Normal donors' PBL was cultured under the same conditions as acute leukemia patients' PBL. A comparison of each group's mean Ei during 3-week culture in rIL 2 is shown in Table 2. Mean values of Ei in cultures derived from AP LAK were similar to N LAK cultures. The mean Ei of cells derived from RP LAK was significantly higher by the third week in culture than that of N LAK (P < .025). This finding, although based on a relatively small population of 12 samples, is encouraging with regard to the feasibility of expansion of cells derived from patients in remission.

<table>
<thead>
<tr>
<th>Patients Studied</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>APs</td>
<td>21 (6 F, 15 M)</td>
</tr>
<tr>
<td>Teste pretreatment</td>
<td>16</td>
</tr>
<tr>
<td>Teste at relapse</td>
<td>5</td>
</tr>
<tr>
<td>RPs</td>
<td>12 (4 F, 8 M)</td>
</tr>
</tbody>
</table>
A comparison of expansion in rIL 2-containing cultures of cells derived from BM and from PBL of the same patients (APs) is shown in Table 3. In four of the six samples cultured, expansion was similar in BM and PB cultures, although longer periods in culture were needed for the BM to reach maximal expansion. In two cases, BM-derived cells did not expand as well as PB-derived cells. This might reflect the higher content of leukemic blasts in the BM cultures or the paucity of LAK precursor cells in BM of patients with active disease.

**Cell surface phenotype of rIL 2-cultured cells.** The data on the cell surface markers of rIL 2-cultured cells in IL 2 indicated considerable variability of the phenotype at different periods of culture in the same individual and between different individuals. Due to this complexity of phenotypic expression, we could not discern a definitive pattern that would distinguish between normal and patients’ cultured cells.

The cultured cells became enriched in cells with LGL morphology, up to a mean of 40% LGLs (range, 15% to 75%), yet this observation was not always matched by markers of NK cells, such as CD16 (Leu 19). Figure 1 shows group data on CD16, NKH-1 marker expression, and on a double-stained population carrying both the T3/NKH-1 phenotype. Data are presented as 75% quartile, median, and lower and upper range.

Cells cultured in rIL 2 were analyzed during 1 to 3 weeks in culture. The distribution of these subpopulations of cells was similar in N LAK and in AP LAK cultures. LAK cells derived from RP LAK had a significantly higher (P < .05) proportion of Leu 11a (CD16)-positive cells than did the N LAK population. A distinct population expressing both T3 and NKH-1 markers was similarly distributed in N LAK and patients’ LAK cells. The distribution of T cell markers on rIL 2-cultured cells is shown in Fig 2. All three groups—N LAK, AP LAK, and RP LAK—showed similar distributions of T cell markers (Fig 2).

The expression of IL 2 receptors (as measured by anti-Tac) on cultured cells from normal donors and from leukemic patients was similarly distributed within the range of 10% to 40%, with median value of 25%. Likewise, no significant differences in the expression of HLA-DR on IL 2-cultured cells were detected between N LAK and patients’ LAK cells: range, 30% to 80%; median value, 58% (results not shown).
We present pooled data for groups of normal individuals and patients, with the main purpose of seeking distinctive features. The individual data accumulated on the normal donors' and patients' LAK phenotype at various times in culture are being analyzed with respect to a possible correlation with functional activity; these results will be reported separately.

Lytic activity of fresh and rIL 2-activated cells against K562 and Raji. The range of cytolytic activity of fresh PBL and rIL 2-cultured cells from normal donors was established against an NK-sensitive K562 target and against the NK-resistant Raji or Daudi cell lines. Preliminary experiments showed similar lytic activity of N LAK cells against Raji and Daudi targets (results not shown).

Thereafter, most assays for LAK activity were performed on Raji cells. In 17 samples of N PBL, no significant cytolytic activity against Raji targets could be detected (mean percentage of lysis at an E:T ratio of 50:1 was 2.9, SD 3.0) (Table 4). Statistical analysis of individual assays showed that any value ≥7% of specific lysis could be considered positive at the P < .01 level of significance (results not shown).

A scattergram of individual assay data, expressed as percentage of specific lysis at an E:T ratio of 50:1 against K562 targets for fresh PBL and for rIL 2-cultured LAK cells from normal donors and acute leukemia patients is presented in Fig 3. The values shown for N LAK cells (five to seven days in culture) and for AP and RP LAK (five to 14 days in culture) represent the highest value on repeated testing. Fresh PBL from normal donors showed NK activity against K562 within the range of 15% to 56%, with a median value of 32%. NK activity of fresh RP PBL was similar to N PBL: median, 34%; range, 5% to 70%. AP PBL had significantly impaired NK lytic activity (P < .001): median, 4%; range, 0% to 34%.

Cytolytic activity of rIL 2-cultured cells from RP LAK was as high as that of N LAK: median of 85% and 82%, respectively, at an E:T ratio of 50:1. Culture in rIL 2 of PBL derived from AP LAK resulted in a significant increase of lytic activity for each individual, at P < .05 to P < .001, yet in ~50% of AP cases, LAK activity on K562 was lower than that of N LAK and RP LAK, with a median value of 42% and a range from 5% to 100%. Most of those with low LAK activity, namely those with values below the normal range, were derived from patients who were dead within 3 months after testing (shaded circles, Fig 3). The follow-up of all patients is too short to allow for a life-table type of analysis for prognostic association of IL 2-activated NK function and prognosis.

LAK activity was also measured against NK-resistant Raji targets (Fig 4, left scale). Although RP LAK showed a high degree of activation, similar to normals, >50% of AP LAK lytic activity was below the normal range, with a median value of 25% lysis at an E:T ratio of 50:1.

Table 4. Mean Lytic Activity of Freshly Isolated and rIL 2-Cultured Cells From the Peripheral Blood of Patients With Acute Leukemia and From Normal Donors

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>K562 Lysis (%)</th>
<th>LU</th>
<th>Raji Lysis (%)</th>
<th>LU</th>
<th>Allogeneic Blasts Lysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N PBL (17)</td>
<td>31.6 (9.9)</td>
<td>79.5 (37.1)</td>
<td>2.9 (3.0)</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>N LAK (18)</td>
<td>76.2 (18.9)</td>
<td>1,890.0 (1,150.0)</td>
<td>75.1 (18.2)</td>
<td>591.0 (384.0)</td>
<td>27.6 (17.1)</td>
</tr>
<tr>
<td>AP PBL (20)</td>
<td>6.2 (8.1)</td>
<td>13.1 (20.2)</td>
<td>0.7 (1.7)</td>
<td>0</td>
<td>0.05* (0.07)</td>
</tr>
<tr>
<td>AP LAK (21)</td>
<td>49.2 (32.8)</td>
<td>664.0 (133.3)</td>
<td>36.9 (32.9)</td>
<td>363.0 (770.0)</td>
<td>18.4 (16.4)</td>
</tr>
<tr>
<td>RP PBL (11)</td>
<td>28.2 (19.2)</td>
<td>74.5 (76.7)</td>
<td>7.6 (8.1)</td>
<td>0</td>
<td>0.47 (0.34)</td>
</tr>
<tr>
<td>RP LAK (12)</td>
<td>84.3 (12.3)</td>
<td>2,491.0 (1,960.0)</td>
<td>64.7 (22.6)</td>
<td>639.0 (513.0)</td>
<td>25.7 (12.4)</td>
</tr>
</tbody>
</table>

Data are expressed as mean lytic activities: as percentage of specific lysis at E:T ratio of 50:1 and in LU/10^5. SD of the means given in parentheses. Mononuclear cells tested were of normal controls (N PBL and N LAK), active ANLL patients (AP PBL and AP LAK), and remission patients (RP PBL and RP LAK).

*Mean of eight tests.
†Mean of six tests.
Although some correlation was observed between grave prognosis and low response to rIL2 in terms of enhancement of NK activity against K562, this correlation was not so pronounced when LAK activity was compared against NK-resistant Raji targets (Fig 4, shaded circles; patients dead within 3 months after testing).

In conclusion, NK activity of MNC of APs was functionally impaired as compared with lytic function of Ns and RPs. The lytic function could be fully restored into the normal range in ~50% of these patients and partially in the remaining half of APs by in vitro culture in 1,000 U/mL rIL2.

Lytic activity of rIL2-cultured cells against fresh/cryopreserved leukemia blasts. The definition of LAK effectors against various allogeneic blasts was based mainly on their functional activity, namely, rIL2-activated cells that will lyse in vitro fresh/cryopreserved (noncultured) tumor cells. In this study of activation of MNCs derived from acute leukemia patients, it seemed important to compare LAK activity as measured on an NK-resistant cell line, eg, Raji, to lytic activity against fresh/cryopreserved leukemic blasts. Figure 4 shows individual assays of N LAK, AP LAK, and RP LAK on Raji cell line and on allogeneic leukemic blasts.

Because baseline NK activity against leukemia blasts was undetectable (Table 4), any value ≥7% was considered positive (P < .005). A panel of ten blast cell preparations from various patients was screened as targets for N LAK cells; each effector was tested on at least two different targets. Although the sensitivity of various blasts to killing by N LAK differed, these differences were not significant by paired Student's t test and most of the results were overlapping. In presenting the results, we pooled all assays of N LAK effectors against various allogeneic blasts.

As shown in Fig 4, the median percentage of lysis of allogeneic blasts by N LAK (at an E:T ratio of 50:1) was 25%, ranging from 2% to 46%. This range of target sensitivity was lower than that for Raji cells (median value, 79%; ranging from 55% to 100%). A similar range of lysis of leukemic blasts was found for LAK cells derived from AP and RP patients. The biologic significance of this is not fully understood, since no correlation between lytic activity against leukemia blasts and the patients' prognosis could be discerned at this point.

The question of ANLL blast targets' sensitivity to LAK cells and of possible ways of enhancing this sensitivity is currently being explored. Results of several experiments are shown in Table 5 and indicate that treatment of leukemic blasts with NA renders them more amenable to lysis by LAK cells, but not by fresh PBL. Fresh normal PBL treated likewise by NA and used as control targets for lysis by LAK did not become more sensitive to LAK. These results imply that the moieties present on leukemic tumor cells but not on normal PBL are recognized by activated killer cells. It also provides a more sensitive assay for assessing the levels of LAK activity against leukemic target cells.

![Fig 3. Scattergram of individual cytolytic assays against an NK-sensitive target (K562), expressed as percentage of specific lysis at an E:T cell ratio of 50:1. Effectors from left to right: PBL, peripheral blood lymphocytes from normal donors (N PBL); active disease patients' (AP PBL); remission patients' (RP PBL); LAK, lymphokine activated killer cells from N, AP, and RP.](image)

![Fig 4. Scattergram of individual cytolytic assays against NK-resistant targets: Raji cell line (left) and fresh/cryopreserved leukemic blasts (right), expressed as percentage of specific lysis at an E:T ratio of 50:1 of rIL2-activated cells (LAK) from N, AP, and RP (details in legend to Fig 3).](image)
Fig 5. Scattergram of individual cytolytic assays of LAK cells derived from APs against autologous (left column) and allogeneic (right column) leukemic blasts. Cells of patients no. 12 and 16 were retested against autologous blasts after these patients achieved remission (shaded circles).

Table 5. Sensitivity of NA-Treated AML Blasts to Killing by rIL 2-Activated Cells

<table>
<thead>
<tr>
<th>Effectors</th>
<th>Experiment No.</th>
<th>Target: AML Blasts</th>
<th>untreated Lysis (%)</th>
<th>NA-Treated Lysis (%)</th>
<th>untreated Lysis (%)</th>
<th>NA-Treated Lysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N PBL</td>
<td>9</td>
<td>1</td>
<td>0.1</td>
<td>0.6</td>
<td>1.2</td>
<td>0.6</td>
</tr>
<tr>
<td>N LAK†</td>
<td>8</td>
<td>10.2</td>
<td>39.6</td>
<td>39.6</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>AP LAK</td>
<td>6</td>
<td>1.0</td>
<td>24.9</td>
<td>24.9</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>N PBL</td>
<td>29</td>
<td>0.3</td>
<td>1.0</td>
<td>1.0</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>N LAK</td>
<td>25</td>
<td>8.8</td>
<td>31.2</td>
<td>31.2</td>
<td>6.4</td>
<td>6.4</td>
</tr>
<tr>
<td>AP LAK auto</td>
<td>30</td>
<td>4.4</td>
<td>12.5</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>AP LAK allo</td>
<td>30</td>
<td>8.2</td>
<td>21.2</td>
<td>21.2</td>
<td>4.8</td>
<td>4.8</td>
</tr>
<tr>
<td>N PBL</td>
<td>41</td>
<td>1.2</td>
<td>0.5</td>
<td>0.5</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>N LAK</td>
<td>40</td>
<td>16.5</td>
<td>56.2</td>
<td>56.2</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>RP LAK auto</td>
<td>32</td>
<td>4.2</td>
<td>12.6</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>RP LAK allo</td>
<td>32</td>
<td>6.5</td>
<td>34.6</td>
<td>34.6</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>N PBL</td>
<td>46</td>
<td>4.4</td>
<td>5.8</td>
<td>5.8</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>N LAK</td>
<td>45</td>
<td>39.8</td>
<td>84.6</td>
<td>84.6</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>N LAK</td>
<td>44</td>
<td>14.7</td>
<td>35.7</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>AP LAK</td>
<td>42</td>
<td>16.1</td>
<td>36.6</td>
<td>36.6</td>
<td>5.7</td>
<td>5.7</td>
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</table>

NA (Boehringer Mannheim) was used at a concentration of 0.02 U/2 to 5 x 10^6 blast cells. Underlined numbers indicate that percentage of lysis differed significantly, as determined by comparison of individual raw cpm data, by paired t test (P < .05).

*Percentage of specific lysis at E:T ratio of 50:1.
†rIL 2-activated cells (LAK).

Figure 5 compares lytic activity of AP LAK against autologous and allogeneic blasts. Although few individual differences in response were observed (some trend for higher lytic function against allogeneic than autologous blasts), the general pattern of lytic activity was similar and not significantly different. Two patients (patients no. 12 and 16) were tested against autologous blasts pretreatment and again when they achieved remission (dark circles, Fig 5). In one of these patients, the response to autologous blasts was significantly enhanced at remission. Numerical data on lytic units/10^7 against K562, Raji, and leukemia blasts are given in Tables 3 and 4.

Overall, these results indicate that testing of lytic activity of LAK cells from leukemic patients on the Raji cell line gives a similar pattern of response as that seen with noncultured blast targets and can be used as a measure of rIL 2 activation in vitro. The majority of patients' LAK cells can kill allogeneic and autologous leukemic blasts, although the sensitivity of blasts is lower than that of Raji/Daudi cell lines.

Kinetics of total lytic activity recovered after in vitro culture of patients' MNCs with rIL 2. Peak lytic activity of patients' LAK cells was achieved in most cases during 2- to 3-week culture (Table 4), whereas the peak of cell expansion occurred at the third week (Table 2). Calculation of TLU/culture (LU x Ei) showed no loss of lytic activity was observed during 3 weeks in culture, as measured by cytolytic activity against K562 or Raji cells (Table 4). Highest lytic activity against leukemic blasts was observed later, namely during the second and third week in AP cultures (AP LAK) compared with that of remission patients' cells (RP LAK).

Thus, the feasibility of in vitro rIL 2 activation of leukemic patients' killer cells and their significant expansion during 3-week culture with good recovery of lytic activity will allow design of a clinical protocol for adult acute leukemia patients.

DISCUSSION

The main objective of this study was to establish the feasibility of ex vivo IL 2-mediated activation of lymphoid cells from adult ANLL patients as a basis for a clinical approach of adoptive immunotherapy. In contrast to the abundance of reported data regarding in vitro activation and
culture of LAK cells from patients with solid tumors, data on LAK cells derived from leukemic patients are scarce.

Impaired NK activity in a group of 20 ANLL patients with AP in comparison with NK activity of normal individuals ($P < .01$) was described in the present study.

Previous studies by Lotzová et al and other researchers demonstrated a deficiency in NK cell activity in adult patients with acute leukemia. The nature of LAK progenitors is still controversial, although most recent data indicate that LAK cells are derived from NK cells with large granular lymphocytes (LGLs) or LALs (large agranular lymphocytes) morphology and carry the CD3−/CD16+ phenotype. The feasibility of in vitro IL 2 activation and expansion of lymphoid cells derived from adult ANLL patients whose initial NK activity was low was an important step toward its possible clinical application. This finding that NK deficiency can be restored by in vitro culture with IL 2 was reported by us and further explored in the present study.

The major new finding of this study is that fresh PBL derived from acute leukemia patients in remission have normal NK activity, as tested by cytolysis assay on K562. Furthermore, these cells could be efficiently activated by culture with rIL 2, with LAK cells showing killer capacity against NK-resistant cell lines (Raji/Daudi) and against fresh/cryopreserved leukemic blasts. These RP LAK cells could be expanded, without loss of lytic capacity, up to 30-fold during 3-week culture. This latter finding differs somewhat from results of other investigators, who found that optimal lytic activity of LAK cells from leukemic patients was achieved after five-day culture with rIL 2 and decreased after 2 to 4 weeks. Culture conditions described by those authors were different from ours; eg, cell density was $2 \times 10^5$/mL culture, whereas our cells were cultured at $5 \times 10^5$ to $10^6$/mL; they used rIL 2 at 2,500 U/mL (Shionogi), whereas ours was at 1,000 U/mL (Cetus).

Normal donors’ LAK cells, cultured by us under the same conditions as patients’ LAK cells, showed peak lytic activity during the first week in culture with a tendency to decreased lytic capacity after 2-week culture (results not shown). The possible explanation of this discrepancy between normal donors’ and patients’ peak lytic activity may be inherent in the in vivo augmentation of NK activity or enrichment of LAK precursor cells in patients who achieved remission of their disease. Cultures derived from remission patients had a significantly higher proportion of CD16+ (Leu 11a)-positive cells than did normal donors’ cultured cells or cultures derived from APs (Fig 2).

Activation of lymphoid cells derived from APs presents many problems: PB or BM samples contain varying proportions of leukemic blasts. Despite additional separation procedures for enrichment of MNCs, all samples cultured in rIL 2 were mixtures of various proportions of blasts and MNCs. Therefore, the culture conditions for AP-derived cells differed from those of RP, in which the initial number of MNC was constant and calculation of E/I presented no problem. The mixed blasts + MNC cultures more resemble tumor infiltrating lymphocytes (TILs) than PBL cultures. Although the initial total number of cells per milliliter was constant, the ratio of blasts to MNCs varied. Therefore, the numerical data on expansion of LAK cells from AP are less accurate than for normal or RP-derived cultured cells.

Previously reported studies addressed this issue, showing that the scarcity of MNCs in mixed cultures was not the reason for impaired NK activity of such preparations since enrichment and readjustment of MNC concentration to normal levels did not restore NK activity to normal values.

In this study, we examined the possibility of activation and expansion of killer cell populations in culture with rIL 2 in the presence of blast cells. Despite initially low NK activity of APs, culture in rIL 2 induced high cytolytic activity in ~50% of cases, as examined on NK-sensitive (K562), NK-resistant (Raji), and leukemic blast targets.

The sensitivity of ANLL blasts to killing by normal and leukemic patients’ LAK cells was lower than that of NK-resistant cell lines like Raji or Daudi (Fig 4) when compared at the same E:T ratio of 50:1 or in terms of LU/10$^7$ (Tables 4 and 5). Although the magnitude of cytolysis was less, the general pattern of lytic activity against allogeneic blasts was similar to that against Raji cells. Testing LAK activity of leukemic patients lymphocytes routinely on Raji/Daudi cell lines may provide a good indicator of the state of LAK activation. A similar finding was reported by other researchers: A statistically significant correlation was shown between lysis of Raji cells and autologous blasts by LAK cells from leukemic patients. Lytic activity of AP LAK against autologous v allogeneic blasts did not show a statistically significant difference in terms of mean percentage of specific lysis. Individualy, however, ~50% of the patients’ LAK cells showed significantly higher response to allogeneic than to autologous blasts ($P < .01$); the other half showed similar or lower values (not significant).

The phenomenon of relatively low sensitivity of leukemic blasts to killing by LAK is not well understood. The correlation between in vivo response to LAK plus IL 2 therapy and in vitro sensitivity of tumor cells to lysis by LAK cells has not been established for individual tumors, although it is empirically implicated.

The lower specific lysis of leukemia blasts by LAK, as described in this study, when compared with solid tumor cells does not necessarily imply low in vivo response to therapy by LAK + IL2. Furthermore, as shown by Lotzová et al, leukemia blasts can be destroyed by IL 2-activated cells by mechanisms other than direct cytolysis measured by four-hour $^{51}$Cr release assay, such as growth inhibition. Our observation of destruction of blast cells in mixed cultures of leukemia blasts and rIL 2-activated lymphoid cells, after 1- to 2-week culture, whereas the same culture without IL 2 then showed an abundance of viable blasts, strengthens the notion that other immunologically mediated processes may be active in tumor cell destruction in vivo and in vitro.

The significant increase in susceptibility of NA-treated blasts to killing by LAK but not by fresh PBL may provide an alternative more sensitive assay system for assessment of LAK activity against leukemia blasts. NA treatment selectively enhances sensitivity of leukemia blasts to killing by LAK, whereas normal PBL treated by NA does not become sensitive to LAK. This observation suggests that LAK recognition structures on leukemic blasts may be partially hid-
den.” It may provide an important tool in determination of tumor cell surface structures that are active either at the binding or the lysis-triggering level.

The results of this study suggest that it is feasible to culture LAK cells from about a one-half of APs (pretreatment), and it may be applicable to a clinical situation of early relapse patients (such as patients no. 12 and 31) in whom the proportion of blasts in the peripheral blood is low and LAK cells can be induced by IL 2 activation, expanded and used in therapy. The more attractive alternative for clinical application is the possibility of using MNCs derived from RPs, because these cells responded so well to IL 2 stimulation, and of cryopreserving the cells and culturing them in IL 2 when patients relapse.

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Interleukin 2 induction of lymphokine-activated killer (LAK) activity in the peripheral blood and bone marrow of acute leukemia patients. I. Feasibility of LAK generation in adult patients with active disease and in remission

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