Systemic Interleukin-2 and Adaptive Transfer of Lymphokine-Activated Killer Cells Improves Antibody-Dependent Cellular Cytotoxicity in Patients with Relapsed B-Cell Lymphoma Treated with Rituximab


Abstract

Purpose: Murine models have shown that antibody-dependent cellular cytotoxicity (ADCC) can be improved with addition of lymphokine-activated killer (LAK) cells to monoclonal antibodies. A pilot trial of rituximab and LAK cells in patients with rituximab-refractory CD20+ lymphoma was conducted to evaluate this approach.

Experimental Design: Ten patients received 3 million units/m² of interleukin-2 (IL-2) i.v. qd on days 1 to 5 and leukapheresed on days 8, 9, and 10. The leukapheresis product was cultured with IL-2 for 48 h to produce LAK cells. Patients then received 375 mg/m² i.v. rituximab and LAK cells on days 10, 11, and 12. The patients also received 3 million units/m² of IL-2 i.v. for 5 days starting day 10. For safety purposes, the first three patients did not receive any LAK cell infusions.

Results: The LAK cell infusions improved the ADCC activity of peripheral blood lymphocytes compared with pretreatment activity and prevented the decline in ADCC seen after infusion of rituximab alone. Therapy was well tolerated and the most clinically significant toxicities were fever and fatigue. Two patients achieved a partial remission and five had stable disease.

Conclusions: The results from these studies suggest that the addition of LAK cells to rituximab augments ADCC in patients with rituximab-refractory lymphoma.

Rituximab is a chimeric anti-CD20 monoclonal antibody (mAb) with human γ-1 and κ constant regions and murine variable regions (1). In vitro, rituximab has significant antilymphoma activity. The underlying mechanisms that account for the antilymphoma activity of rituximab include antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity, and direct induction of apoptosis (2, 3). Most of these in vitro studies were conducted using cell lines, and the potential contributions of these specific mechanisms in vivo are unclear. Studies in animal models using complement-deficient or complement-depleted hosts have suggested that the primary mechanism of in vivo tumor lysis induced by mAbs involves ADCC rather than complement-dependent cytotoxicity (4). Lending support to the notion that ADCC is an important mechanism of action of rituximab in vivo are clinical trials showing differential outcome dependent on distinct Fc receptor polymorphisms with differential affinities for the immunoglobulin Fc domain (5–7).

Engineering mAbs to be more effective in the induction of ADCC is an area of active research (8). However, potent ADCC requires competent host cellular effector mechanisms. ADCC, which is carried out by several distinct subsets of effector cells, including CTLs and natural killer (NK) cells, has been reported to be suboptimal in patients with cancer (9, 10). One approach to improve effector mechanisms and enhance ADCC activity is to activate NK cells and CTLs. For instance, the culture of peripheral blood lymphocytes (PBL) with interleukin-2 (IL-2) activates a subset of killer cells. This population of lymphokine-activated killer (LAK) cells is composed of mostly NK cells and CTLs with both acting as effector cells (11). Although initially promising, the infusion of LAK cells has largely been abandoned, as clinical trials failed to show efficacy, especially in lymphoma patients where few or no responses were seen (12–14).

Although the adoptive transfer of LAK cells in non-Hodgkin's lymphoma patients has had minimal effect in vivo when used by itself, it is unknown whether its effects are different in the setting of bound antibody. Numerous laboratory studies suggest that the addition of LAK cells improves the efficacy of tumor-specific mAbs. LAK cells are

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known to express human Fc receptors and can mediate ADCC (11, 15, 16). In a murine lymphoma model, the combination of in vitro–activated LAK cells and tumor-specific anti-Thy-1.1 IgG2a mAb had significantly greater antitumor activity than mAb therapy alone. Moreover, the combination of LAK cells and mAb was also superior to IL-2 and mAb therapy (17). Other investigators have shown similar results combining mAb therapy and LAK cells in various tumor cell lines and murine models for tumors ranging from lymphoma to colon cancer (18–25). Together, these results support the hypothesis that the addition of IL-2 and LAK cells to chimeric anti-CD20 antibodies may result in accentuated ADCC activity and possibly induce greater lymphoma cell death than that seen with anti-CD20 therapy alone.

To further explore the role of ADCC mechanisms in lymphoma patients given rituximab, the capacity of PBLs from patients to induce ADCC was examined before and after treatment with rituximab in correlation with the clinical trial population. The results from these studies suggest that ADCC activity in the lymphoma patients was inferior compared with normal controls. Moreover, ADCC activity decreased after the infusion of rituximab. These findings led us to conduct a pilot study to evaluate whether ADCC can be augmented by the adoptive transfer of LAK cells and subsequently enhance antilymphoma effects of rituximab in patients with non–Hodgkin’s lymphoma.

Materials and Methods

**Patient eligibility.** PBL samples from eight patients with low-grade or mantle cell lymphoma were used in the preclinical study. These patients were concurrently enrolled in a clinical trial evaluating the addition of rituximab to autologous peripheral blood stem cell transplantation and thus had the good organ function and performance status required to receive high-dose therapy.

In the clinical trial, 10 patients with a diagnosis of CD20+ B-cell non–Hodgkin’s lymphoma were enrolled. All patients had been previously treated with rituximab and were considered refractory to this therapy (achieved less than a partial remission or progressed within 6 months of rituximab therapy). All patients enrolled met eligibility criteria, including preserved organ function defined as a left ventricular ejection fraction ≥45%, serum creatinine ≤2.0 mg/dL, total bilirubin ≤2.0 mg/dL, forced expiratory volume in 1 s, forced vital capacity, and diffusing capacity of the lung for carbon monoxide ≥75% of predicted values; absolute neutrophil count ≥1 × 10⁹/L; and platelets ≥75 × 10⁹/L. Patients were excluded for any of the following: HIV infection, central nervous system involvement by lymphoma at the time of enrollment, receipt of organ allografts or allogeneic stem cell transplant, and previous exposure to i.v. IL-2. The use of steroids or other immunosuppressive medications as a premedication to rituximab or at any other time was not allowed.

All participating patients signed informed consent before enrolling in these studies in accordance with current Food and Drug Administration, National Cancer Institute, state, federal, and institutional regulations.

**Study design.** In the initial preclinical study, ADCC was measured from heparinized blood drawn from patients receiving rituximab for in vitro purging purposes during stem cell mobilization for use in peripheral blood stem cell as described previously (26). These patients received 375 mg/m² rituximab 3 days before receiving cyclophosphamide, which was used to mobilize the hematopoietic stem cells. ADCC samples were drawn from these patients at baseline 1 h after completion of rituximab infusion and on day 3 before receiving cyclophosphamide. Peripheral blood from 10 normal volunteers served as controls.

The clinical trial was a two-part study of patients with relapsed CD20+ B-cell lymphoma. Part one of the trial consisted of three patients treated with i.v. IL-2 and rituximab but received no LAK cell infusions. The purpose of this first part was to assess feasibility and safety of the combination of IL-2 and rituximab given in this format. The remaining patients proceeded to part two and were treated with i.v. IL-2, rituximab, and LAK cell infusions (Fig. 1). All patients received 3 million units/m² IL-2 by 24-h continuous i.v. infusion on days 1 to 5 and again on days 10 to 14. Approximately 36 h following the completion of the first 5 days of IL-2 infusion (days 8, 9, and 10), patients underwent 3 consecutive days of leukapheresis for PBL collection. Patients required either a large bore peripheral or central venous catheter. Leukapheresis was done using a Cobe Spectra (Lakewood, CO) apheresis machine. The goal for each leukapheresis collection was 5.0 × 10¹⁰ mononuclear cells, but any amount collected was processed. The number of circulating absolute lymphocytes was checked and recorded before each daily leukapheresis session to assess degree of lymphocytosis from the IL-2 treatment. Likewise, the numbers of mononuclear cells collected per daily leukapheresis session were recorded. Serial monitoring of serum calcium was done and supplemental calcium was given as needed.

Following the final leukapheresis, 375 mg/m² i.v. rituximab was given followed 1 h later by infusion of LAK cells i.v. over 30 min on days 10, 11, and 12. Note that the three patients in part one did not undergo leukapheresis or receive any infusions of LAK cells.

**Summary of LAK cell generation.** This procedure was done with good laboratory practice with appropriate documentation in a clinical graft engineering laboratory that is Joint Commission on Accreditation of Healthcare Organizations and American Association of Blood Banks accredited.

The PBLs collected from each session were cultured in vitro in the setting of high-dose IL-2 for the generation of LAK cells as described previously (27). Briefly, cells were cultured in serum-free medium (X-VIVO10, BioWhittaker, Walkersville, MD) with glutamine, streptomycin, gentamicin, and 1,000 units/mL IL-2 at a concentration of 2.5 × 10⁶ to 5.0 × 10⁹ cells/mL. Cells were cultured in 1,000 mL aliquots in 3 L LifeCell bags (Nexell, Inc., Irvine, CA) at 37°C for 2 days in a Stericult Tissue Culture Incubator (ThermoQuest Corp., Austin, TX). After incubation, cells were washed, concentrated, and suspended in a

![Fig. 1. Treatment schema. IL-2 (3 million units/m²) was infused by 24 h continuous i.v. infusion on days 1 to 5 and again on days 10 to 14. On days 8, 9, and 10, patients underwent leukapheresis for the production of LAK cells. Following the final leukapheresis, 375 mg/m² i.v. rituximab was given followed 1 h later by infusion of LAK cells i.v. over 30 min on days 10, 11, and 12. Note that the first three patients did not receive any infusions of LAK cells.](image-url)
Cell count was adjusted to 3×10^6 with 5% culture medium, and resuspended with culture medium. Placed in glass culture tubes (Kimble, Vineland, NJ) measuring 6 cm. The samples were counted in a gamma counter (Packard Instrument, Waltham, MA), and percentage specific lysis was calculated as follows: minimum release − 100 µL targets + 100 µL culture medium → harvest 100 µL supernatant; maximum release was obtained by adding 1 mol/L HCl to each well, refluxing, and then harvesting 100 µL from each well. The ADCC assay was done in a similar manner to above. However, the CD20+ SB cell line was used as the target. Target cells were incubated on ice for 30 min after 51Cr incubation with or without 50 µg rituximab or PBS-A1x control.

**Cytotoxic in vitro efficacy and ADCC determination of LAK cells and PBLs.** Cytotoxic in vitro efficacy of LAK cells was tested using the 51Cr release assay against NK-resistant Raji tumor cell lines and NK-sensitive human erythroblasts (K562) cell lines. Effector cells were obtained directly from the LAK product (2×10^7 cells) or extracted from a heparinized whole blood sample (20 cm³). Effector cells were isolated using Ficoll-Hypaque, washed twice with RPMI 1640, and resuspended in culture medium (RPMI 1640 + 10% FCS). Cell concentration was adjusted to 3×10^5/mL. Tumor cell lines were incubated with 250 µCi 51Cr for 1 h at 37°C in H₂O bath, washed thrice with 5% culture medium, and resuspended with culture medium. Cell count was adjusted to 3×10^4 targets/mL. E:T ratios were as follows: 100:1, 50:1, 25:1, 12.5:1, 6.25:1, 3.13:1, 1.57:1, and 0.78:1. All determinations were done in six replicates. Plates were incubated for 4 h at 37°C, 5% CO₂ humidified atmosphere. Plates were centrifuged for 5 min at 1,000 rpm, and 100 µL of supernatant were harvested and placed in glass culture tubes (Kimble, Vineland, NJ) measuring 6×50 mm. The samples were counted in a gamma counter (Packard Instrument, Waltham, MA), and percentage specific lysis was calculated as follows: minimum release − 100 µL targets + 100 µL culture medium → harvest 100 µL supernatant; maximum release was obtained by adding 1 mol/L HCl to each well, refluxing, and then harvesting 100 µL from each well. The ADCC assay was done in a similar manner to above. However, the CD20+ SB cell line was used as the target. Target cells were incubated on ice for 30 min after 51Cr incubation with or without 50 µg rituximab or PBS-A1x control.

**Results**

As part of the initial preclinical study, ADCC of PBLs was measured in 8 patients and 10 controls (Fig. 2). Similar

### Table 1. Toxicities according to National Cancer Institute Common Toxicity Criteria

<table>
<thead>
<tr>
<th>Study</th>
<th>Toxicities</th>
<th>IL-2</th>
<th>Leukapheresis</th>
<th>LAK cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gr 2</td>
<td>Gr 3</td>
<td>Gr 4</td>
</tr>
<tr>
<td>Part 1 (3 Pts)</td>
<td>GI/liver</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pulmonary</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cardiac</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Renal/FEN</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Constitutional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heme</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part 2 (7 Pts)</td>
<td>GI/liver</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pulmonary</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cardiac</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Renal/FEN</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Constitutional</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heme</td>
<td>2</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

NOTE: Recorded by grade and as attributed to IL-2, leukapheresis, or LAK cell infusions. Numbers correspond to the number of events recorded. Abbreviations: Gr, grade; Pts, patients; GI, gastrointestinal; FEN, fluids, electrolytes, nutrition; Heme, hemolytic.
results were seen at various E:T ratios, and data from the 40:1 ratio are shown. Mean specific lysis was 37.9% (range, 23.9-51.8%) in patients and 59.7% (range, 52.4-67%) in controls (P = 0.0035). The patient baseline ADCC dropped to 5.5% (range, 0-11.1%) 1 h after rituximab was infused (P = 0.0002). ADCC improved at 24 h (mean, 28.1%; range, 10.6-45.6%; P = 0.32; Fig. 2).

In the clinical study, 10 patients (median age, 63; range, 46-66) with a median of 6 (range, 3-10) prior therapies were enrolled. Histology was seven follicular center cell, two small lymphocytic, and one diffuse large cell. All patients were refractory to rituximab.

The median mononuclear cell collection for each of three consecutive leukapheresis products was 5.49 × 10^10, 6.22 × 10^10, and 2.73 × 10^10, respectively. These cells were subsequently used for the creation of LAK cells. The median numbers of LAK cells per infusion were 1.96 × 10^10, 1.68 × 10^10, and 0.56 × 10^10, respectively. The median total of 3.52 × 10^10 (range, 1.70 × 10^10 to 8.72 × 10^10) LAK cells was infused per patient.

Three patients were entered onto part one of the study and tolerated therapy relatively well. The most clinically significant toxicities were fever and fatigue. Transient grade 2 and 3 anemia and thrombocytopenia were noted in patients with baseline cytopenias, resolving once IL-2 infusion was discontinued. Seven patients were entered onto part two of the study, which included leukapheresis and LAK cell infusions. One patient was withdrawn from study therapy before receiving LAK cells due to elevation of liver transaminases from the IL-2. The remaining patients all had similar toxicities as observed in part 1 of this trial, mostly attributed to the IL-2 infusions with the exception of higher-grade hematotoxicity requiring transfusions of platelets and RBCs mostly as a consequence of the serial leukapheresis. LAK cell infusions were very well tolerated, except for one patient who had a grade 3 allergic-type reaction during each infusion. Toxicity was transient and resolved shortly after infusion. Toxicities in all patients attributable to each component of their treatment are outlined in Table 1. One patient received steroids at another institution and was not evaluable for response. Of the remaining eight evaluable patients, two patients achieved a partial remission and four patients had stable disease. The median duration of response was 163 days (range, 137-498 days; see Table 2).

Flow cytometry measuring CD45, CD20, CD3, CD4, CD8, and CD56 was used to assess the composition of lymphocytes in samples from peripheral blood and LAK products. The treatment as given achieved the expected expansion of T lymphocytes and NK cells while suppressing B lymphocytes. The NK cell compartment at all time points was increased when compared with baseline values before any study therapy (median NK cells/mm^3 at baseline or at days 0, 8, 9, 10, and 30 were 81, 301, 162, 130, and 328, respectively; P = 0.19). Interestingly, the absolute numbers of NK cells seemed to decrease as a result of leukapheresis in preparation for LAK cells, but rebounded following completion of study treatment, as evidenced by the 1-month evaluation point (Fig. 3). These data indicate that the use of moderate dose IL-2 and adoptive transfer of LAK cells as given in this study resulted in the acute and prolonged expansion of the NK cell population.

### Table 2. Patient characteristics and results of all patients enrolled

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age</th>
<th>Histology</th>
<th>No. prior treatments</th>
<th>Best response</th>
<th>PFS (d)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>65</td>
<td>FML</td>
<td>6</td>
<td>SD</td>
<td>498</td>
<td>No LAK cells given</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>SLL</td>
<td>4</td>
<td>PR</td>
<td>—</td>
<td>No LAK, lost to f/u</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>FML</td>
<td>10</td>
<td>SD</td>
<td>168</td>
<td>No LAK cells given</td>
</tr>
<tr>
<td>Part 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>66</td>
<td>FML</td>
<td>7</td>
<td>SD</td>
<td>163</td>
<td>LAK cells given</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>FSC</td>
<td>9</td>
<td>—</td>
<td>—</td>
<td>Not evaluable, received steroids</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>FML</td>
<td>6</td>
<td>—</td>
<td>—</td>
<td>Not evaluable, no LAK cells given, unable to tolerate IL-2</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>DLC</td>
<td>5</td>
<td>PD</td>
<td>N/A</td>
<td>LAK cells given</td>
</tr>
<tr>
<td>5</td>
<td>53</td>
<td>CLL</td>
<td>3</td>
<td>PR</td>
<td>137</td>
<td>LAK cells given</td>
</tr>
<tr>
<td>6</td>
<td>61</td>
<td>FML</td>
<td>3</td>
<td>PD</td>
<td>N/A</td>
<td>LAK cells given</td>
</tr>
<tr>
<td>7</td>
<td>66</td>
<td>FSC</td>
<td>4</td>
<td>SD</td>
<td>161</td>
<td>LAK cells given</td>
</tr>
</tbody>
</table>

Abbreviations: PFS, progression-free survival; FML, follicular mixed lymphoma; SLL, small lymphocytic lymphoma; FSC, follicular small cleaved; DLC, diffuse large cell; CLL, chronic lymphocytic leukemia; SD, stable disease; PR, partial response; PD, progressive disease; N/A, not applicable.
transcript levels were markedly decreased (14-fold compared to pretreatment levels. On day 12, the levels of Foxp3 mRNA transcript levels were dramatically decreased (>150-fold) compared with pretreatment levels. Interestingly, CD4 mRNA transcripts as an indicator of CD4 T cells did not significantly change over these time intervals (Fig. 6B). On the other hand, there was a significant drop in CD8 mRNA transcript levels, suggesting a reduction in the CD8 T-cell compartment (Fig. 6C). Virtually identical results were observed in two other patients.

The results of the FcRIIa-131H/R polymorphism revealed two patients to be homozygous for R/R and one patient was heterozygous for H/R. The results of the FcγRIIA-158V/F polymorphism revealed two patients to be heterozygous for F/V and one patient was homozygous for V/V.

LAK cells were activated appropriately by our approach and were able to carry out specific lysis of both NK-sensitive erythroleukemia K562 and NK-resistant Raji tumor cell lines. In addition, LAK cells were more cytotoxic than the PBLs collected from the same patient before incubation with IL-2. At an E:T ratio of 12.5, specific lysis of the SB20 cell increased from 4.3% with PBLs to 36.5% (P = 0.01) with LAK cells. Furthermore, LAK cells were more potent in carrying out ADCC with rituximab against the CD20+ SB cell line than PBLs (42% LAK cells versus 11% PBLs; P = 0.03).

The ability of PBLs to carry out ADCC at various times during the study treatment was determined by measuring the percentage specific lysis of a CD20+ SB cell line in the setting of rituximab at an E:T ratio of 12.5. The percentage specific lysis at baseline, following 5 days of IL-2 but before any LAK cell infusions, following LAK cell infusions, and day 30 was 8.75, 11.4, 23.2, and 13.1, respectively (Fig. 4). The highest ADCC activity corresponded to PBLs collected soon after infusion of LAK cells, with the change from baseline reaching near statistical significance (P = 0.08). The measured ADCC activity after IL-2 only and at day 30 was higher than baseline, but the difference was not statistically significant (P = 0.23 and 0.44, respectively).

Furthermore, the ADCC activity of PBLs in relation to first rituximab exposure is depicted in Fig. 5. The percentage specific lysis for the two time points before rituximab exposure (baseline and after 5 days of IL-2) is 8.75 and 11.4, respectively. The percentage specific lysis for the three time points following the first rituximab exposure (24 h, 48 h, and 3 weeks after first rituximab) is 11.2, 23.2, and 13.1, respectively. No decline in ADCC activity was seen following rituximab exposure. The trend toward increased ADCC activity following rituximab exposure did not reach statistical significance.

To test whether expansion of regulatory T cells played a role in the ADCC activity observed, the results of a representative experiment evaluating Foxp3 gene expression are illustrated in Fig. 6A. Evaluation of mRNA transcript levels revealed that at day 10 there was a slight increase (4-fold) compared with pretreatment levels. On day 12, the levels of Foxp3 mRNA transcript levels were markedly decreased (14-fold compared with pretreatment levels and >40-fold versus day 10 levels.) At day 30, Foxp3 mRNA transcript levels were dramatically decreased (>150-fold) compared with pretreatment levels. Interestingly, CD4 mRNA transcripts as an indicator of CD4 T cells did not significantly change over these time intervals (Fig. 6B). On the other hand, there was a significant drop in CD8 mRNA transcript levels, suggesting a reduction in the CD8 T-cell compartment (Fig. 6C). Virtually identical results were observed in two other patients.

The results of the ADCC activity following rituximab seem to be an important mechanism of action of rituximab in patients with B-cell malignancies. Unfortunately, the results from the preclinical studies suggest that ADCC is suboptimal in patients with non–Hodgkin’s lymphoma. Moreover, infusion of rituximab actually significantly decreases ADCC activity at least in the short term. In light of these findings, strategies aimed at optimizing ADCC in rituximab-treated patients become more important.

It is not clear why rituximab causes a transient decrease in ADCC activity in PBLs from patients with non–Hodgkin’s lymphoma. One possible explanation is that rituximab causes saturation or depletion of the effector cells that mediate ADCC following infusion of the antibody. Interestingly, a shift of the immunophenotype of PBLs after rituximab was not observed. On the other hand, this decrease in ADCC activity following administration of rituximab was not observed in the setting of IL-2 and LAK cell infusions (Fig. 5). In fact, the expansion of NK cells with i.v. IL-2 and the infusion of LAK cells actually may have increased levels of ADCC activity following rituximab exposure.

Studies in animal models have shown that the combination of mAb, LAK cells, and IL-2 is far more effective compared with each agent used alone. The present studies confirm the efficacy...
of this approach in patients with lymphoma. NK cells can be effectively expanded in vivo by the administration of i.v. IL-2 given at moderate doses. IL-2 at moderate doses is well tolerated and induces a rapid and durable expansion of the NK cell component of PBLs as has been reported by others with similar or alternate IL-2 dosing schedules (27, 30–32). The NK expansion occurred quickly after only 5 days of i.v. IL-2 exposure and was most pronounced at 1 month persisting 2 to 3 weeks following cessation of IL-2 treatment as depicted in Fig. 3. The absolute NK numbers were transiently decreased around the day 9 and 10 evaluations, presumably as a result of the leukapheresis procedures on days 8, 9, and 10. Notwithstanding, even after leukapheresis, the absolute numbers of NK cells were still higher than those seen at baseline before any IL-2 exposure.

Using specific lysis of a CD20+ SB cell line with and without rituximab, the ability of PBLs from this expanded population to act as effector cells and mediate ADCC was assessed. The results of the present studies show a minor increase in ADCC activity after exposure to IL-2. In addition, there was also a significant increase in ADCC activity after in vitro culturing of these effector cells with IL-2 to induce LAK cells, findings that were similar to previous studies in vitro (33). Interestingly, PBLs collected from patients after reinfusion of LAK cells were more potent mediators of ADCC than PBLs collected from patients after exposure to IL-2 but before the reinfusion of LAK cells. These data suggest that LAK cells not only can mediate ADCC but also may enhance total ADCC activity in vivo over and above the activity provided by exposure to IL-2 alone. This supports the hypothesis that augmentation of ADCC can be provided by adoptive transfer of LAK cells.

Other schedules or routes of administration of IL-2 may also improve the efficacy of rituximab. S.c. IL-2 increases the absolute number of NK cells, resulting in increased ADCC activity (34). This expansion is clearly dose related and appeared in patients receiving daily s.c. IL-2 and in patients receiving thrice weekly s.c. IL-2. Similarly, when we superimpose ADCC activity and absolute number of LAK cells, we see an increase in ADCC corresponding to expansion of NK cells as a result of increasing absolute numbers of NK cells after IL-2 exposure. This increased activity is still evident by the day 30 evaluation time point (Fig. 7). Interestingly, the highest levels of ADCC activity were not seen when the highest numbers of NK cells were detected in the peripheral blood. Rather, the highest levels of ADCC activity corresponded to the time shortly after adoptive transfer of LAK cells. These observations suggest that, although the quantity of effector cells is important, the functional capacity of these cells to mediate ADCC is more important. This has been previously raised in several other studies, indicating that, although low doses of IL-2 increase the number of NK cells, intermediate doses of this cytokine improve the function of these cells (35). Furthermore, expansion of regulatory T cells may also play an important role in suppression of ADCC (36). In the patients evaluated in the clinical study, there seemed to be a slight expansion measured following IL-2 infusion, but paradoxically, a sustained suppression was observed after infusion of LAK cells. Likewise, FcyRIIA and FcyRIIIA polymorphisms have been associated with improved modulation of ADCC and subsequent response to rituximab in patients with lymphoma (5–7) but not chronic lymphocytic leukemia (37). In three patients tested, only one contained the V/V homozygous polymorphism of FcyRIIA that would predict for a better clinical response to rituximab. This patient's clinical response was stable disease, and the ADCC activity was similar to the other two patients tested (data not shown). Unfortunately, PBLs were not available to do these studies on all patients. Although these
results may be important in the interpretation of the clinical response, they do not explain the rise in ADCC activity seen after LAK cell infusion over baseline and IL-2 alone using each patient’s PBLs as controls. In a larger cohort of patients, it would be interesting to see if increasing the number of effector cells, such as done here, with LAK cell infusions has a more robust effect in patients with FcγR polymorphisms predicting a stronger ADCC response.

Given the small numbers of patients in this pilot study, the therapeutic clinical utility of this treatment regimen should be interpreted with caution. Nonetheless, the stabilization and partial responses seen in this heavily pretreated and rituximab-refractory population are encouraging. The use of i.v. IL-2 as used in this pilot study to expand the NK cell population and to induce LAK cells requires that PBLs be collected with the in vitro induction of LAK activity followed by infusion in relatively short order. Perhaps, the use of alternate preparations, such as prolonged administration of s.c. IL-2, would allow for adequate expansion and collection of PBLs to induce LAK cells over a much longer period (34, 35, 38). These LAK cells could then be infused weekly or monthly to take full effect of the more standard dosing of rituximab and maximize exposure to LAK cells that mediate ADCC more effectively than “unprimed” PBLs. It is difficult to compare the results of this study to approaches using IL-2 alone (34, 35, 38). Patients enrolled in these studies are very different, although each study is fundamentally evaluating whether rituximab efficacy can be improved by augmenting ADCC activity in the patient. Importantly, each of these studies had encouraging enough results to continue the development of the regimen.

Rituximab was given on 3 consecutive days to each patient in this study to accommodate the logistical issues previously discussed and to maximize exposure to LAK cells. No unexpected toxicities were observed with more frequent doses of rituximab similar to data previously reported (39). Furthermore, the development of alternative methods of adoptive transfer of effector cells that is less cumbersome and/or dependent on patient scheduling may allow for a more flexible and more conventional dose scheduling.

In vitro and animal data suggest that augmentation of ADCC results in greater tumor lysis by mAbs. In this pilot study, we show that ADCC activity can be augmented by adoptively transferring in vitro–generated LAK cells and may overcome resistance to anti-CD20 mAb therapy in patients with non–Hodgkin's lymphoma who are rituximab refractory. Further studies will be needed to fully evaluate the efficacy of this approach.

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References

29. Miura Y, Thoburn CJ, Bright EC, Chen W, Nakao S, Hess AD. Cytokine and chemokine profiles in autologous

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graft-versus-host disease (GVHD): interleukin 10 and interferon γ may be critical mediators for the development of autologous GVHD. Blood 2002;100:2690–9.  
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