Lymphokine-Activated Killer Cells From Normal and Lymphoma Subjects Are Cytotoxic for Cells Coated With Antibody Derivatives Displaying Human Fcγ


Lymphokine-activated killer (LAK) cells were successfully generated in all cases from blood mononuclear cells obtained from six patients with NHL. The LAK cells from three of these patients and from five normal adult donors were tested for their effector abilities in antibody-dependent cellular cytotoxicity (ADCC) against guinea pig leukemic lymphocytes coated with various antiidiotype antibodies. Cells from all the donors behaved similarly. Mouse monoclonal antibodies of IgG1, IgG2a, and IgG2b isotypes invoked no ADCC. However, substantial ADCC was invoked by the chimeric antibody FabFc, in which Fab′γ from mouse antiidiotype is thioether-bonded to human normal Fcγ. Similar results were obtained on testing LAK cells from a normal donor against uncultured human lymphoma targets coated with native or chimeric antiidiotype. The ADCC invoked by the mouse-human chimeric antibodies appears to depend on the human Fcγ they display and not on the univalency of the derivatives used. The findings imply that LAK technology could usefully augment serotherapy that uses antibody derivatives displaying human Fcγ.

HUMAN LYMPHOCYTES activated in culture by interleukin-2 (IL-2) for 48 hours or longer acquire a broad cytotoxic potential that sometimes encompasses autologous tumor cells. Strengthen efforts are being made to exploit this phenomenon therapeutically.

The lymphokine-activated killer (LAK) cells appear predominantly to arise from lymphocytes described variously as null (ie, non-B, non-T), large granular, or natural killer (NK)—the classes defined by each of these three designations showing at least an extensive overlap. It should be noted that NK targets are much more restricted than LAK targets, although this might result from the activation by IL-2 raising cytotoxic performance above previously low levels, rather than permitting attacks on completely new targets. A minor contribution to LAK activity appears to derive from T cells rendered broadly cytotoxic in culture.

We shall accept the view (summarized in reference 7) that large granular lymphocytes (LGL) are a distinct class distinguished by morphology, surface markers that include the Fcγ-receptor CD16, and the two cytotoxic activities NK and LAK. A third cytotoxic role attributed to them is that of the principal lymphocytic effector, or K cell, in antibody-dependent cell-mediated cytotoxicity (ADCC). Here target cells are marked for destruction by a coating of IgG antibody, which adheres to the Fcγ-receptor of LGL. Consistent with this activity of LGL, murine LAK cells have recently been shown to be active in ADCC.

The cells of non-Hodgkin’s lymphoma (NHL) present an attractive target for LAK cell therapy, being disseminated and often having ready access to the blood. In the clinical study cited, two of 108 patients receiving LAK cells had NHL. Both underwent remissions, one complete and one partial.

We have been interested for some years in treating NHL with antiidiotype antibody (anti-Id) or its derivatives. Early therapeutic efforts have given variable results, commonly a partial remission induced by infusion of up to several grams of antibody. Among the difficulties that have emerged are modulation of surface idiotype, mutation of the idiotype, and an uncertainty about the roles of the various effectors in eliminating the antibody-coated neoplastic cells. The last of these problems impedes therapeutic strategy: it is not clear whether one should be trying to invoke complement or ADCC or scavenging by macrophages to clear the cells.

Ready means of enhancing the activities of these effectors have not been available. Now, however, LAK cell technology promises to enhance ADCC and might thereby usefully complement the infusion of antibody.

This report describes the ability of LAK cells, from normal or NHL-bearing subjects, to kill lymphoma cells coated with anti-Id. Among the antibodies used is the chimeric derivative FabFc, where Fab′γ from mouse monoclonal anti-Id is thioether-bonded, hinge to hinge, to human normal Fcγ. This derivative is univalent, thereby avoiding rapid antigenic modulation, and possesses human instead of mouse Fcγ so as to prolong metabolic survival, reduce immunogenicity, and better recruit human effectors. Present evidence that LAK cells are strongly and consistently effective in ADCC when presented with human Fcγ on the target cells.

MATERIALS AND METHODS

Patients and LAK cell generation. Six patients with NHL were chosen for study. They had all presented with follicular center cell lymphoma (FCC), and none was receiving chemotherapy at the time of sampling for LAK cell generation. All gave informed consent according to guidelines of the local Ethical Committee after being informed of the procedures and attendant risks. Normal donors were healthy laboratory personnel in the age range of 21 to 55 years. Blood was collected into preservative-free heparin and separated in Ficoll-Hypaque. Cells collected at the interface were washed with Eagle’s minimal essential medium (MEM) containing heparin (20 U/mL) and counted. They were then resuspended in flasks at 1.5 × 10^6/mL in RPMI 1640 medium containing glucose (200 mmol/L), pyruvate (100 mmol/L), penicillin and streptomycin.

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Submitted March 9, 1988; accepted July 29, 1988.

Supported by Tenovus and the Cancer Research Campaign.

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Table 1. Generation of LAK Cells From Patients With B-Cell Lymphoma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease Type* and Status</th>
<th>Tumor Cells in Blood (% of Indicated Mononuclear Count)</th>
<th>% Specific Lysis (±SD) of Indicated Targets at Indicated Effector:Target Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>K562</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40:1</td>
</tr>
<tr>
<td>S.A.</td>
<td>CB/CC, nodular</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>M, 72</td>
<td>Partial remission for 2 yr post chemotherapy</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>N.L.</td>
<td>CC, diffuse</td>
<td></td>
<td>53% of 7200/μL</td>
</tr>
<tr>
<td>M, 60</td>
<td>Progressive on intermittent chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.R.</td>
<td>CB/CC, nodular</td>
<td></td>
<td>50% of 3400/μL</td>
</tr>
<tr>
<td>F, 55</td>
<td>Partial remission for 2 yr postantibody†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.H.</td>
<td>CC, diffuse</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>M, 57</td>
<td>1 mo postchemotherapy</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>J.R.</td>
<td>CB/CC, nodular</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>M, 55</td>
<td>Slowly progressive on intermittent chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.R.</td>
<td>CB/CC, nodular</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>M, 76</td>
<td>Partial remission for 5 yr postchemotherapy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Incubation time, four hours.
Abbreviations: CB, centroblastic; CC, centrocytic; ND, not determined.

*Kiel classification.25†Univalent chimeric (mouse/human) antiidiotype.16

(100 IU/mL), fungizone (2 μg/mL) (conditioned medium [CM]), and 10% decomplemented human AB serum, to which 10 U/mL of recombinant human IL-2 (donated by DuPont, Glenolden, PA) had been added. Cells were incubated for three to five days at 37°C in an incubator with 5% CO2. Activated cells were collected by centrifugation and suspended in medium for cytotoxicity assays.

Target cells. The human erythroleukemia cell line K562 and two human B-lymphoblastoid cell lines, Daudi and Raji, were cultured in CM containing 10% fetal calf serum (FCS). They were used directly from culture. Fresh uncultured tumor targets consisted of patients’ tumor cells obtained from blood or involved lymph node, either at presentation or during disease exacerbation (Table 2). Cells were prepared, washed, and cryopreserved16; the proportion of tumor cells in the preparations was estimated from the surface Ig phenotype obtained by immunofluorescence. Target B cells from the guinea pig L2C leukemia were obtained fresh from blood of terminal animals as described previously.20

Monoclonal antibodies. Mouse monoclonal anti-Id specific for surface Ig on the guinea pig L2C leukemia were raised as previously described.21 Anti-Id(1) and anti-Id(2) are of isotypes IgG1 and IgG2α, and display KA for reaction with surface Ig at 37°C of 1.1 x 105 mol/L1 and 2.3 x 105 mol/L1, respectively.22 Anti-Id(1), of isotype IgG2b, was raised against the L-chain of L2C Ig, which can be harvested from urine of leukemia-bearing animals.23 It recognizes idiotypes expressed on both the free L-chains and the IgMα synthesized by the tumor, and it fails to react with normal IgM. At concentrations above 5 μg/mL, all three of these antibodies saturate the L2C surface Ig (1.3 to 1.6 x 105 molecules/cell).24

Mouse monoclonal antibodies against idiotypic and isotypic determinants on patients’ tumor cells (N.L. and A.H.) were raised using as antigen the IgMα rescued from the tumor cells by xenohybridization.24 Anti-Id for each patient, both of isotype IgG1, were selected on the basis of specific binding to tumor-derived IgM in the presence of normal human serum (50%). Specific binding to the tumor cells

Table 2. Human Lymphoma Cells Used as Uncultured Targets

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease Type*</th>
<th>Source of Cells</th>
<th>Surface Ig Isotype† (Density, % Cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.A.‡</td>
<td>CB/CC, nodular</td>
<td>Lymph node</td>
<td>IgMDδ (77)</td>
</tr>
<tr>
<td>M, 69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N.L.</td>
<td>CC, diffuse</td>
<td>Blood</td>
<td>IgMDδ (90)</td>
</tr>
<tr>
<td>M, 60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.R.</td>
<td>CB/CC, nodular</td>
<td>Blood</td>
<td>IgMDδ (85)</td>
</tr>
<tr>
<td>F, 52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.H.</td>
<td>CC, diffuse</td>
<td>Blood</td>
<td>IgGk (90)</td>
</tr>
<tr>
<td>M, 57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.H.</td>
<td>CB/CC, terminal blastic</td>
<td>Blood</td>
<td>IgMα (90)</td>
</tr>
<tr>
<td>M, 72</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CB, centroblastic; CC, centrocytic.

*Kiel classification.25
†Surface Ig was detected by immunofluorescence. Levels of fluorescence are indicated by plus signs, and the percentages of positive cells in the preparations are in parentheses.
‡The first four patients also appear in Table 1 as donors of LAK cells. Ages given are at the time of donating tumor or LAK cells.
was confirmed by immunofluorescence. An IgGl antibody to human
Ce was obtained during the generation of the anti-Id, being selected
by specific reactivity with IgM preparations on ELISA.

*Polyclonal antibodies.* Rabbit antiserum specific for Lc surface
idiotype was raised against Fabα isolated from a papain digest of
the tumor cell surfaces. The IgG fraction, prepared by precipita-
tion with ammonium sulphate and DEAE-cellulose chromatogra-
phy, was absorbed with guinea pig normal Ig to yield polyclonal
anti-Id. From this the Fab/c derivative was prepared by limited
digestion with papain.

*Chimeric antibodies.* These were constructed chemically.
FabIgG, in which Fab’γ from mouse antibody is thioether-bonded to
human normal IgG, was prepared as described previously. FabFc
derivatives (Fig 1) were prepared using Fab’γ from mouse mono-
clonal IgGl antibody and Fc from human normal IgG. F(αb’γ)2 was
derived by peptic digestion with IgGl 18 mg/mL, pepsin (product
P6887, Sigma Chemical Co, St Louis) 0.3 mg/mL, pH 4.2, 37°C.
Progress was monitored by passing aliquots through Zorbax GF250
column, (Pharmacia Inc. Piscataway, NJ) 0.3 mg/mL, 2-mercaptoethanol
0.1 mg/mL, 2-mercaptoethanol (product
P3125, Sigma) 1 mmol/L, pH 7.4,
37°C, 60
minutes. The 50-Kd fraction of the digest (Fab’γ plus Fcγ) was
separated on Sephacryl S200 and then passed onto DEAE-Trisacryl
in 20 mmol/L TrisHCl, pH 8.0. All Fabγ passes through the DEAE
column, since the starting basic IgG lacks the more acidic Fabγ
fractions, while the retarded Fcγ remains to be eluted with 0.5
mol/L TrisHCl, pH 8.0. The Fcγ thus prepared is essentially
entirely from IgG1: IgG2 is relatively resistant to cleavage under the
digestion conditions used, IgG3 yields a large Fc fragment separated
on the Sephacryl, and IgG4 is not present in significant amount in
the basic IgG.

FabFc can now be prepared by (1) reducing F(ab’γ)2 to mono-
meric form with free sulphydryl (SH) groups; (2) allowing the Fabγ
to react with a large molar surplus of o-phenylenediamidemaleimide
(Sigma) to yield Fab’γ with free maleimide groups; (3) allowing the
Fab maleimide groups to react with SH of reduced Fc. In more
detail, the F(ab’γ)2 was reduced with 10 mmol/L 2-mercaptoethanol
at pH 8.0 and then separated on Sephadex G25 (Pharmacia) in 10
mmol/L disodium EDTA, pH 4.6. To the eluted protein was added
an equal volume of 4 mmol/L o-phenylenediamidemaleimide in 40%
(vol/vol) dimethylformamide, 60% 50 mmol/L sodium acetate, pH
5.3. The reaction was allowed to proceed for 30 minutes at 5°C. The
protein was then separated by binding to the cation-exchange resin
Phospho-Ultrogel (LKB), from which it was eluted with 0.5 mol/L
sodium acetate, pH 5.3. The number of maleimide groups per
molecule (obtained by adding a standard solution of 2-mercapto-
ethanol and back-titrating SH groups with 2,2’-dithiopyridine) was
0.84 to 1.05, suggesting that where two vicinal SH groups occur, the
bismaleimide compound links them intramolecularly. The Fab γ
was then added to Fcγ that had earlier been reduced (10 mmol/L
dithiothreitol, pH 8.0), separated on Sephacryl S200, concentrated
to about 10 mg/mL in an Amicon Ultrafiltration cell (PM10
membrane), and kept in a nitrogen atmosphere at pH 5.3. The molar
to FabFc was 1:2.5. The protein mixture was incubated at 20°C
for two hours before separating the FabFc product on Sephacryl
S200 (Fig 1). Residual SH groups within the Fc hinge (theoretically,
three of the original four remain) were encouraged to reform at least
one of the two original hinge region disulfide bonds by undergoing
disulfide exchange with 1 mmol/L 2,2’-dithiopyridine (Alidrich,
Gillingham, Dorset, UK) at pH 5.3. A cycle of absorption elutin
on protein A-Sepharose (Pharmacia) got rid of a small amount of
contaminating Fab dimer. The only contaminant then detectable
was Fc dimer (<10%). Recovery of antibody Fab’γ in the FabFc is
usually 50% to 60%.

*Measurements of cytotoxicity.* Target cells were used direct
from culture (K562, Raji), fresh from the blood of leukemia-bearing
guinea pigs (Lc), or immediately after thawing (lymphoma cells
from patients). Cells (~3 x 10⁶, 100 µL of phosphate-buffered saline
(PBS), and 100 µL of Na; CrO₄ (Amer-
sham International, UK) was added. Incubation was for one hour at
37°C, followed by three washes with PBS and resuspension at
10⁵/mL in CM with 10% FCS.

For LAK cytotoxicity (in the absence of antibody), target cells
were added, at 10⁶ cells in 100 µL, to wells containing 100 µL
CM/FCS and effector cells in numbers
specified for individual
experiments. Cultures were in triplicate in round-bottom microtiter
plates unless indicated otherwise. After incubation for four hours at
37°C in 5% CO₂, the plates were centrifuged at 175 g for five
minutes and the supernatants harvested. Released isotope was
measured in a γ-counter with maximum release estimated
by incubation of target cells with
0.5% Nonidet P40. Supernatant release was determined
by incubation of target cells with CM/FCS only. The percent
specific lysis was calculated as

\[
\text{Percent specific lysis} = \frac{\text{maximum cpm} - \text{spontaneous cpm} \times 100%}{\text{experimental cpm} - \text{spontaneous cpm}}
\]

For ADCC, a fixed antibody concentration (20 µg/mL) and
effector:target ratio (25:1) were used, after preliminary titrations
had shown these values to achieve plateau levels of cell lysis (Fig 2).
Labeled target cells (10⁶) in 50 µL CM/FCS were treated with 100
**RESULTS**

**LAK cells from patients with lymphoma.** The disease status for each of the six patients is indicated in Table 1: none had rapidly progressive tumor at the time of study. As judged by the killing of either K562 or Daudi cells, blood mononuclear cells from all the patients generated good LAK activity (Table 1). It is notable that this ability persisted in N.L. and B.R., both of whom had a substantial proportion of tumor cells among the blood mononuclears. Normal adult donors generated LAK activity against K562 cells at a comparable level: 74% ± 10% (SD) specific lysis with an effector:target ratio of 20:1.

LAK cells from four of the patients were also tested against cryopreserved autologous tumor cells. In two of the four cases, a modest degree of lysis was effected (Table 1).

**ADCC mediated by LAK cells against a xenogeneic target.** The guinea pig L2C leukemia, maintained by continual passage in vivo, proved a convenient model for assessing the ADCC capability of LAK cells: the L2C cells label well, are relatively insensitive to human LAK effectors in the absence of antibody, and have available against them a well characterized panel of mouse monoclonal anti-Id.

Results obtained using cells from five normal donors and three subjects with lymphoma are shown in Table 3. Four of the five normal subjects yielded a small degree of LAK activity, i.e., nonantibody-dependent lysis, while two of the three lymphoma subjects yielded substantially more. There was never any significant increase in lysis when native mouse antibody coated the cells; indeed, in the majority of determinations the lysis achieved was less, and on occasion notably so. The picture was entirely different when the chimeric FabFc, displaying human Fcγ1, coated the cells: here in all instances there was a striking increase in the extent of lysis achieved.

To assess whether a minor mouse IgG subclass, or a combination of subclasses, could be effective in mediating ADCC, IgG from a pooled mouse antiserum to L2C surface Ig was included among the antibodies in Table 3. No lysis above that occurring in the absence of antibody was observed. It should be noted, in view of recent reports that mouse IgG3 can mediate ADCC with human LGL, that we do not know what proportion of our IgG preparation was represented by this minor subclass.

In addition to using chimeric antibodies containing human Fcγ1, we have found that human LAK cell preparations regularly mediate a good level of ADCC against L2C cells in the presence of rabbit polyclonal anti-Id (Table 4), rabbit polyclonal anti-μ (data not shown), and guinea pig polyclonal anti-major histocompatibility complex (MHC) class II (raised in strain 13 guinea pigs against strain 2 lymphocytes; data not shown).

**ADCC mediated by LAK cells against human tumor targets.** LAK cells from a normal donor were arrayed against allogeneic lymphoma cells from two patients in the presence of various antibodies (Table 5). Negligible cytotox-
LAK CELLS AND ANTIBODY DISPLAYING HUMAN Fcγ

icity was seen in the absence of specific antibody and in the presence of specific monoclonal anti-Id of mouse isotype IgG1. However, in the presence of univalent chimeric derivatives containing Fab′γ from specific anti-Id and displaying human Fcγ, the LAK cells effected modest but significant killing over the three-hour incubation period.

Role of antibody valency in mediating ADCC. The fact that the univalent chimeric antibodies examined here can invoke significant ADCC, while the parent mouse IgG1 antibodies do not, could be due to the chimeric derivatives possessing a human Fcγ and/or to their being univalent. Previously we have reported that univalency of antibody possessing a human Fcγ and/or to their being univalent. invoke significant ADCC, while the parent mouse IgGl antibodies do not, could be due to the chimeric derivatives containing human Fcγ. However, it was possible to compare the lysis invoked by rabbit IgG antibody and its Fc-containing derivative Fab/c, in the knowledge that rabbit Fcγ can recruit human lymphocytic effectors.34

In Table 4 it is seen that human LAK cells achieved good levels of lysis of L2C leukemic cells during a three-hour incubation in the presence of either IgG or Fab/c anti-Id. Despite the fact that the univalent derivative was consistently the more effective, the results leave no doubt that bivalency alone does not rule out substantial ADCC by LAK cells.

Role of CD16. The fact that LAK-cell ADCC is dependent on use of the Fcγ-receptor CD16 (FcRIII) was confirmed by the inhibition shown by antibody 3G8, directed against this receptor.31 Table 6 shows the diminished efficiency of a three-hour ADCC occurring after preincubating the effectors with anti-CD16. Preincubation with control antibody had no significant effect. The inhibition was obvious but required higher concentrations of anti-CD16 than were needed for comparable inhibitions of the ADCC mediated by unstimulated blood lymphocytes.

DISCUSSION

No strict definition of LAK cells has been proffered. However, it appears that the broad, non-MHC-restricted, nonantibody-dependent cytotoxicity that is the LAK hallmark requires that mononuclear cells be activated by IL-2 in

Table 3. Activity of LAK Cells Against Antibody-Coated L2C Leukemic Lymphocytes

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Isotype</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
<th>S.A.</th>
<th>N.L.</th>
<th>B.R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td></td>
<td>0.6 ± 0.4</td>
<td>6.8 ± 0.5</td>
<td>6.4 ± 2.6</td>
<td>8.8 ± 1.0</td>
<td>8.2 ± 2.6</td>
<td>36 ± 7</td>
<td>0.3 ± 1.6</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Anti-Id (1)</td>
<td>Mouse IgG1</td>
<td>2.9 ± 0.7</td>
<td>7.4 ± 2.3</td>
<td>2.4 ± 1.8</td>
<td>3.8 ± 1.0</td>
<td>7.2 ± 0.5</td>
<td>26 ± 2</td>
<td>-1.6 ± 1.1</td>
<td>9.9 ± 2.0</td>
</tr>
<tr>
<td>Anti-Id (2)</td>
<td>Mouse IgG2a</td>
<td>0.1 ± 0.5</td>
<td>10 ± 1</td>
<td>2.8 ± 5.2</td>
<td>3.7 ± 0.9</td>
<td>8.5 ± 0.7</td>
<td>ND</td>
<td>-0.8 ± 0.8</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>Anti-Id (11)</td>
<td>Mouse IgG2b</td>
<td>ND</td>
<td>2.1 ± 1.6</td>
<td>3.1 ± 3.4</td>
<td>2.9 ± 1.6</td>
<td>2.9 ± 1.6</td>
<td>ND</td>
<td>-0.2 ± 0.9</td>
<td>4.7 ± 1.6</td>
</tr>
<tr>
<td>Polyclonal</td>
<td>Mouse Fab′γ1-human Fcγ1</td>
<td>ND</td>
<td>ND</td>
<td>5.4 ± 1.8</td>
<td>ND</td>
<td>8.3 ± 1.0</td>
<td>ND</td>
<td>-0.6 ± 1.1</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>Anti-Id (A.H.)</td>
<td>Mouse Fab′γ1</td>
<td>ND</td>
<td>ND</td>
<td>59 ± 3</td>
<td>46 ± 6</td>
<td>39 ± 3</td>
<td>64 ± 4</td>
<td>16 ± 0.3</td>
<td>79 ± 4</td>
</tr>
</tbody>
</table>

Effect:target ratio is 25:1. Incubation time is three hours.

Abbreviation: ND: not determined.

* N1 through N5 are normal subjects; S.A., N.L., B.R. are subjects with lymphoma.

Table 4. Activity of LAK Cells Against L2C Leukemic Lymphocytes Coated with Bivalent or Univalent Anti-Id

<table>
<thead>
<tr>
<th>Antibody</th>
<th>% Specific Lysis Given by LAK Cells From Indicated Donor*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N3</td>
</tr>
<tr>
<td>Bivalent (rabbit IgG)</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>Univalent (rabbit Fab/c)</td>
<td>44 ± 0.4</td>
</tr>
</tbody>
</table>

* Designated as in Table 3, where the levels of LAK cytotoxicity effected by cells from these donors in the absence of antibody are also set out.

Table 5. Activity of LAK Cells From a Normal Donor Against Antibody-Coated Human Lymphoma Cells

<table>
<thead>
<tr>
<th>Antibody†</th>
<th>Isotype</th>
<th>% Specific Lysis (±SD) Against Indicated Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Id (N.L.)</td>
<td>Mouse IgG1</td>
<td>3.6 ± 3.8</td>
</tr>
<tr>
<td>Anti-Id (A.H.)</td>
<td>Mouse IgG1</td>
<td>0.6 ± 4.0‡</td>
</tr>
<tr>
<td>FabFc anti-Id (N.L.)</td>
<td>Mouse Fab′γ1-human Fcγ1</td>
<td>14 ± 4.6</td>
</tr>
<tr>
<td>FabIgG anti-Id (A.H.)</td>
<td>Mouse Fab′γ1-human IgG</td>
<td>2.0 ± 2.8‡</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

* Male, age 29 years.
† Mouse monoclonal anti-Id prepared against idiotypic IgM from either N.L. or A.H. and chimeric antibodies prepared from them. In the FabIgG derivative, the human IgG is used simply because of the Fcγ it displays.
‡ Anti-Id (N.L.) and its chimeric derivative showed no binding whatever to A.H. cells, and vice versa (examinations by flow cytofluorimetry), so that the indicated tests represent negative results for LAK-type cytotoxicity.
culture for at least 48 hours; and it seems that the sole or principal LAK progenitors are LGL. Morine LAK cells so defined are efficient in ADCC, and in this report we describe similar behavior by their human counterparts. There are to hand consistent reports that ADCC due to human LGL can be augmented by brief exposure of the effectors to IL-2 and that ADCC can be effected by IL-2-dependent cloned LGL lines.

The major finding to emerge from the present work is the efficiency with which LAK cells lyse targets coated with antibody that displays human Fcγ, even over as short a period as three hours (Table 3). The actual human isotype involved is undoubtedly Fcγ1, which accounts for more than 90% of the Fc in FabFc (see Materials and Methods). Chimeric antibodies containing mouse variable regions and human Fc can be synthesized either by chemical means or by genetic engineering. Among the advantages envisaged for them is efficient recruitment of human effectors (complement, macrophages, K cells), an expectation confirmed here for ADCC by LAK cells.

No LAK donor has been found to yield cells that fail to effect ADCC when presented with human Fcγ on their targets. Apparently the variability in ADCC performance among LAK cells from different donors is less than the well-known variability in the performance of unstimulated blood lymphocytes. It is also reassuring to note that all six lymphoma patients tested were able to generate active LAK cells (Table 1), even when the presence of tumor cells in the blood suggested extensive marrow infiltration.

Although LAK cells have characteristically been used after culture for three to five days in IL-2, there is a case for looking at their performance in ADCC at earlier times, as the LAK activity of CD16-positive cells has been reported to peak after culture for only one to two days, although with some variation for different cell targets. A reduction in the necessary period of culture would clearly be of great technical convenience.

We have not observed any significant ADCC effected by LAK cells against targets coated with the commonly occurring mouse monoclonal isotypes IgG1, IgG2a, and IgG2b. There are contradictory reports as to the abilities of the progenitor lymphocytes, unstimulated or exposed briefly to IL-2, to effect ADCC via the various mouse IgG isotypes. The discrepancies are difficult to evaluate because of variables additional to antibody isotype: the nature of target cell and surface antigen, the antibody affinity, and the possible presence of monocytes among mononuclear effectors kept only briefly in culture. However, a persuasive case has now been made for the ability of human LGL to kill targets coated with the rarest mouse IgG isotype, IgG3. It is a reasonable expectation then that LAK cells will also use this isotype. We saw no ADCC when using a mouse polyclonal IgG preparation, but its content of IgG3 is likely to have been <5%.

Using rat monoclonal antibodies, the isotype IgG2b stood out as the only one capable of invoking ADCC with human lymphocytic effectors, so again there is an expectation that this isotype will invoke ADCC by LAK cells. From our own and others' experience we can now summarize the minimum ADCC capability of human LAK cells: (1) A demonstrated ability to lyse cells coated with antibody displaying human Fcγ1; (2) An expected ability to lyse cells coated with mouse IgG3 or rat IgG2b antibody. There is clearly a need eventually to assess whether these generalizations apply over a range of neoplastic targets, including carcinoma cells.

The addition of autologous LAK cells to antibody therapy, provided an appropriate Fcγ is presented, holds promise of enhancing the attack on tumor cells by both antibody-independent and antibody-dependent means. It can be seen in Table 3 that two of the donors—both, as it happens, subjects with lymphoma—yielded cells that displayed appreciable antibody-independent cytotoxicity, and in the presence of chimeric antibody increased their killing to the highest observed. The major drawback we see is the added difficulty in evaluation when two types of treatment are combined.

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