Identification of a Novel CD56\(^{−}\) Lymphokine-activated Killer Cell Precursor in Cancer Patients Receiving Recombinant Interleukin 2\(^{1}\)

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ABSTRACT

Circulating lymphokine-activated killer (LAK) cell activity in cancer patients receiving recombinant interleukin 2 (rIL-2) therapy is confined to cells expressing the CD56 surface marker. However, CD56\(^{−}\) cells from these patients but not normal individuals have been reported to exhibit LAK cytotoxicity only following in vitro activation with rIL-2. Studies were performed to document the existence of CD56\(^{−}\) LAK precursor cells and to phenotypically characterize this population in patients receiving rIL-2 therapy using fluorescence-activated cell sorter-purified CD56\(^{−}\) cell subsets. Initial studies confirmed that CD56\(^{−}\) cells exhibit NK activity ([20 ± 7 (SE) LU/10\(^{6}\) cells] but not LAK activity (0 ± 0 LU/10\(^{6}\) cells) when evaluated directly from peripheral blood of patients receiving rIL-2. CD56\(^{−}\) cells from patients but not normal individuals developed significant LAK cytolytic activity against NK-resistant COLO 205 targets (16 ± 3 LU/10\(^{6}\) cells) when cultured for 3 days with 1500 units/ml rIL-2. The CD56\(^{−}\) LAK precursor activity was confined to cells expressing a CD56\(^{+}\)CD16\(^{+}\) phenotype and a large granular lymphocyte morphology; little or no NK or LAK precursor activity was detectable in CD56\(^{−}\)CD5\(^{+}\) T-cells from patients. Phenotypic characterization of CD16\(^{+}\)CD56\(^{−}\) cells revealed that this population is uniformly CD11a\(^{+}\), CD18\(^{+}\), and CD38\(^{+}\) and is heterogeneous in its expression of CD11b, CD11c, and CD16/Leu 11c. These results indicate that rIL-2 administration induces enhanced LAK precursor activity in a novel population of CD56\(^{−}\)CD16\(^{+}\) cells.

INTRODUCTION

NK\(^{3}\) cells represent a distinct population of non-T-, non-B-lymphocytes which are capable of mediating non-MHC-restricted cytotoxicity against a limited number of target cells without prior activation. Exposure of NK cells to IL-2 in vitro or in vivo results in the acquisition of LAK cell activity, which is characterized by the acquisition of cytolytic activity against a wider spectrum of target cells than those killed by NK cells as well as elevated cytotoxic activity against NK-sensitive tumor cells (1–3). The ability of IL-2 to induce LAK activity has provided the basis for numerous clinical trials for cancer immunotherapy in which IL-2 was administered alone or in combination with ex vivo activated LAK cells (4, 5).

Although most if not all human NK and LAK cells express the CD56 surface marker, these cells have been shown previously to be phenotypically and functionally heterogeneous (6–8). Distinct subsets of NK cells can be identified based on the qualitative coexpression of CD16 and the quantitative expression of CD56 (7, 9). A majority of peripheral blood cells which mediate non-MHC-restricted cytotoxicity coexpress the CD56 and CD16 surface markers (CD56\(^{+}\)CD16\(^{+}\)). Subsets of NK/LAK cells expressing CD56\(^{+}\)CD16\(^{+}\) or CD56\(^{+}\)CD16\(^{+}\) phenotypes also exist and exhibit distinct functional and proliferative properties following IL-2 activation. Recently, additional subsets of NK cells and NK progenitors have been phenotypically defined in thymic and fetal tissues and suggest that NK cells and T-cells may arise from similar progenitor cells, although their relationships to the mature subsets of NK cells remain to be defined (10, 11).

The ability of IL-2 administration to increase the absolute and relative numbers of CD56\(^{+}\) NK and LAK cells provides a unique opportunity to study the phenotypic and functional heterogeneity of human natural killer cell subsets. Following rIL-2 administration, relative and absolute increases in circulating CD56\(^{−}\) cells occur and are associated with the development of circulating LAK cytotoxicity in this population (6, 12, 13). Although LAK cells induced in vivo are confined to CD56\(^{−}\) cells, we have previously observed that CD56\(^{−}\) cells from patients receiving IL-2 therapy exhibit LAK cytotoxic activity, but only after additional in vitro exposure to rIL-2 (13). In this report we describe the appearance of a novel CD56\(^{−}\) LAK cell precursor in the circulation of patients receiving IL-2 therapy and demonstrate that this activity is mediated by a novel subset of large granular lymphocytes which expresses a CD56\(^{−}\)CD16\(^{+}\) phenotype.

MATERIALS AND METHODS

Cell Lines. The K562 erythroleukemia (NK-sensitive) and COLO 205 (NK-resistant) cell lines were obtained from the American Type Culture Collection (Rockville, MD) and were maintained in RPMI 1640 supplemented with 10% and 20% FCS, respectively.

Patient Population. This study utilized patients with metastatic melanoma or renal cell carcinoma who received rIL-2 under various treatment protocols. Most of the patients studied received rIL-2 as a part of the priming phase of rIL-2/LAK therapy administered by bolus (1 x 10\(^{6}\) units i.v. every 8 h for 5 days) or continuous infusion (3 x 10\(^{6}\) units/m\(^{2}\) i.v. daily for 5 days). Other patients received rIL-2 alone (1 x 10\(^{6}\) units/m\(^{2}\)/day continuous infusion for 5 days) as a part of other treatment regimens. Peripheral blood for use in these studies was obtained 2 to 5 days after the last dose of rIL-2.

Isolation of Peripheral Blood Mononuclear Cells. Heparinized blood from patients or normal volunteers was diluted 1:2 with HBSS, layered onto Ficoll-Hypaque (Organon Teknika Corporation, Durham, NC), and centrifuged (300 x g) for 20 min. PBMCs were collected from the interface and washed three times with HBSS containing 5% FCS prior to use.

Flow Cytometry and Cell Sorting. Two- and three-color immunofluorescence was performed using commercially available monoclonal antibodies specific for CD56 (anti-Leu 19), CD16 (anti-Leu 11a or anti-Leu 11c), CD5 (anti-Leu 1), CD3 (anti-Leu 4), CD4 (anti-Leu 3a,b), CD8 (anti-Leu 2a), CD25, a/ß or y/ß T-cell receptors, CD11a, CD11b, CD11c (anti-Leu M5), CD14, CD18, CD19 (anti-Leu 12), HLA-DR (Becton Dickinson, Mountain View, CA), and MOPC-21 control IgG1 (Sigma Chemical Co, St. Louis, MO). For two-color staining, antibodies directly conjugated with either fluorescein isothiocyanate or phycoerythrin were used. For three-color staining, cells were initially incubated with unconjugated primary antibody, washed, and...
incubated with a biotinylated goat anti-mouse IgG. Cells were subse-
sequently blocked by incubation with saturating amounts of the γ1 my-
eloma protein MOPC 21C and then incubated with additional directly
conjugated primary antibodies and allophycocyanin-conjugated avidin.
Cells were incubated with antibodies on ice for 30 min, and all washes
were performed using 5% newborn calf serum-HBS at 4°C without phenol
red. Cell populations were purified by cell sorting using a FACS 420 or
a FACSStar Plus at rates of 1500 cells/s with abort circuitry activated.

Purity of sorted fractions was routinely greater than 95%, as assessed by
subsequent flow cytometric analysis.

Induction of LAK Cytolytic Activity. Cells were cultured in RPMI
1640 supplemented with 10% FCS, 2 mm L-glutamine, 50 units/ml
penicillin, 50 g/ml streptomycin sulfate, 50 g/ml gentamicin sulfate,
and 1500 units/ml rIL-2 (Cetus Corporation, Emeryville, CA). Cultures
were established at 5 × 10^5 cells/ml in 24 well flat-bottomed plates
(Costar Laboratories, Cambridge, MA). After 3 days at 37°C, cells were
harvested, washed three times in 5% FCS-HBS, and assayed for cyto-
tolytic activity in a ^51Cr release assay.

Depletion of CD56- Cells Using Magnetic Beads. Up to 2 × 10^5
cells were incubated with saturating amounts of anti-CD56 antibody in
a 100-μl total volume in a 96-well plate for 30 min on ice. Cells were
washed in the plate three times with 200 μl 5% newborn calf serum-
RPMI 1640 and resuspended in 100 μl 5% newborn calf serum-RPMI
1640. Goat anti-mouse-coated magnetic beads (Advanced Magnetics,
Cambridge, MA) were added, and the cells were incubated on ice for 30
min prior to the removal of bead-cell complexes with a magnet. The
cells were counted and used directly in a ^51Cr release assay.

Chromium Release Assays. K562 and COLO 205 tumor cell lines
were labeled with 200 μCi ^51Cr (New England Nuclear, Boston, MA)
for 1 h at 37°C and then washed three times in RPMI 1640 containing
10% FCS. Target cells were subsequently incubated for 30 min at 37°C
and washed once before resuspension in medium and plated at 2500
cells/well in 96-well round-bottomed plates (Falcon Labware). Effector
cells were added at various numbers to achieve a range of effector-to-
target ratios. The plates were centrifuged (40 × g for 5 min) and incub-
at at 37°C in a 5% CO₂ incubator. After 4 h, supernatants were
collected using the Skatron supernatant collection system (Skatron,
Inc., Sterling, VA) and assayed for radioactivity by gamma scintillation
counting.

Spontaneous cpm were determined by measuring the amount of ^51Cr
released from 2500 target cells incubated in media for 4 h. Total cpm were
determined by measuring the amount of ^51Cr released from 2500 target
cells incubated with 0.5% Triton-X detergent. Data are presented as
percentage specific ^51Cr release or as LU. One LU is defined as the
number of effector cells required to lyse 30% of the target cells.

RESULTS

Generation of LAK Activity in CD56- Cells from Cancer
Patients Receiving rIL-2. CD56+ and CD56- cells from rIL-
2-treated cancer patients were isolated by cell sorting and evalua-
ted for NK and LAK activity prior to and following in vitro

culture with rIL-2. The CD56- population failed to dem-
strate LAK cytotoxicity against the COLO 205 cell line when
tested immediately following isolation from all (13 of 13) rIL-
2-treated cancer patients tested to date, although this popula-
tion exhibited NK cytotoxic activity against the K562 cell line (Table 1). Following in vitro activation by rIL-2, patient CD56-
cells expressed low but detectable cytotoxicity against COLO
205 target cells, thereby demonstrating the presence of LAK
precursor activity in CD56- cells from rIL-2-treated cancer
patients (Table 1). Furthermore, phenotypic analysis before and
after in vitro rIL-2 revealed that the CD56- population re-
mained CD56- (data not shown), demonstrating that the LAK
activity exhibited by IL-2 activated CD56- cells was not due to
the development or the expansion of a CD56+ subpopulation.

CD56- LAK Precursor Activity Is Mediated by Non-T-Cells.
Inasmuch as T-cells have been demonstrated to possess LAK-
like cytotoxic activity following in vitro exposure to rIL-2,
experiments were performed to determine whether the LAK
precursor activity was mediated by CD56+ T-cells within the
CD56- population (14, 15). CD56- cells were sorted into CD56+ and CD56- subsets and were cultured with rIL-2 (1500
units/ml) for 3 days. CD56- LAK precursor activity was found
predominantly in the CD56- non-T-cell population (Table 2).
Patient CD56-CD5+ cells exhibited minimal or no LAK cyto-
lytic activity following culture with rIL-2. Although low levels
of cytolytic activity were observed in CD56-CD5+ cells in some
experiments and the absolute number of LU recoverable in this
culture could be comparable to absolute LU in the less
frequent CD56-CD5- fraction, the fact that activity in the
CD56-CD5+ subset was not consistently detected in all exper-
iments leaves the significance of the cytolytic activity of this
population open to question (Table 2).

Similar experiments performed using cells from normal indi-
viduals revealed that neither the CD56- T-cell population
(CD56-CD5+) nor the CD56- non-T-cell (CD56-CD5-) pop-
ulation acquired measurable LAK activity following a 3-day
culture with rIL-2, although unseparated PBMCs demon-
strated significant LAK cytotoxicity following in vitro
activation with rIL-2. Thus, the ability of CD56-CD5- cells to
develop LAK cytotoxicity following in vitro activation by rIL-2 is
a unique property of CD56- cells collected from individuals
receiving rIL-2.

CD56- LAK Precursor Activity Is Mediated by CD16+ Lympho-
ocytes. Subsequent experiments were performed to deter-
mine whether the CD56- LAK activity was mediated by ex-
pressing the NK-associated marker CD16+. During therapy with rIL-2, a minor subset of circulating CD56-CD16+ cells
appears and can constitute up to 8% of circulating mononuclear
cells (Fig. 1). CD56-CD16+ and CD56-CD16- cells were iso-
lated from normal individuals and cancer patients receiving
rIL-2 and evaluated for LAK cytotoxicity following 3 days
of activation with rIL-2. Results from these experiments revealed
that the CD56- LAK precursor activity in rIL-2-treated
patients was mediated exclusively by the CD16+ subpopu-
lation, and CD56-CD16- cells showed no detectable LAK ac-
tivity following in vitro activation (Table 3). These data dem-
strate that CD56- LAK precursor activity is confined to
CD56-CD16+ cells and does not represent cytotoxicity by non-
NK-like cells, such as activated monocytes. Furthermore,
CD56-CD16+ and CD56-CD16- cell populations isolated from
normal individuals did not develop LAK cytotoxicity.

<p>| Table 1 Lytic activities of CD56+ and CD56- cells from patients receiving rIL-2 and normal individuals |
|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Cells</th>
<th>Pre* rIL-2</th>
<th>Pre* rIL-2</th>
<th>Pre* rIL-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD56+</td>
<td>28 ± 9</td>
<td>113 ± 16</td>
<td>242 ± 110</td>
</tr>
<tr>
<td>CD56-</td>
<td>0 ± 0</td>
<td>16 ± 3</td>
<td>20 ± 7</td>
</tr>
<tr>
<td>Normal</td>
<td>0 ± 0</td>
<td>81 ± 12</td>
<td>102 ± 35</td>
</tr>
<tr>
<td>CD56+</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>CD56-</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

* Mean ± SEM of four independent experiments.
+ Mean ± SEM of three independent experiments.
** Not determined.
cells exhibit significant NK activity when assayed directly upon effector activity measured directly in patient peripheral blood is mediated exclusively by CD56+ cells. However, CD56~CD16+ cells showed little effect on the cytolytic activity of cultures of patient PBMCs reduced LAK cytolytic activity by 85%, although residual LAK activity was still present (Table 5). Removal of contaminating CD56+ cells from cultures of CD56~CD16+ cells showed little effect on the cytolytic activity expressed (17 LU versus 14 LU). Efficiency of the treatment procedure was documented by a similar treatment of normal IL-2-activated PBMCs, in which complete loss of LAK cytotoxicity was observed following removal of CD56+ cells. This indicates that magnetic bead depletions were capable of completely removing LAK cells from mixed populations in which CD56+ cells were the only LAK effector cell. Furthermore, the fact that depletion of CD56+ cells from patient CD56~ cells had little effect on cytotoxicity provides evidence that the activity exhibited by CD56~ cells is not due to contaminating CD56+ cells.

Additional support for these conclusions was obtained by comparing the number of purified CD56+ cells needed for minimal detectable cytolytic activity and comparing with the numbers of CD56~CD16+ cells required for similar levels of cytotoxicity. The results (Fig. 2) indicate that comparable levels of cytotoxicity are achieved using either CD56~CD16+ cells at E:T ratios of 1.5:1 or purified CD56+ cells at 0.375:1. Had the activity of the CD56~CD16+ population been due to contaminating CD56+ cells, the cytotoxicity observed was not due to contaminating CD56+ cells, CD56~CD16+ cells did not exhibit detectable LAK activity when assessed directly following isolation from peripheral blood, prior to in vitro activation (Table 4). Significant NK activity was, however, observed in the CD56~CD16+ population. These results confirmed our prior observations that LAK effector activity measured directly in patient peripheral blood is mediated exclusively by CD56+ cells. However, CD56~CD16+ cells exhibited significant NK activity when assayed directly upon isolation and develop LAK activity only after in vitro stimulation with rIL-2.

To demonstrate that the cytotoxicity observed was not due to contaminating CD56+ cells, CD56~CD16+ cells were activated with 1500 units/ml rIL-2 for 3 days and treated with anti-CD56 antibody and goat anti-mouse IgG-coated magnetic beads, and residual CD56+ cells were removed by exposure of the suspension to a magnet. The effectiveness of this treatment was monitored by assessing the cytolytic activity of normal PBMCs cultured with IL-2 following a similar treatment with anti-CD56 and magnetic beads. Depletion of CD56+ cells from cultures of patient PBMCs reduced LAK cytolytic activity by 85%, although residual LAK activity was still present (Table 5).

Table 2 Acquisition of LAK activity by CD56~CD5+ and CD56~CD5+ subsets from rIL-2-treated patients following in vitro activation with rIL-2

<table>
<thead>
<tr>
<th>Cell source</th>
<th>PBMCs</th>
<th>CD56~CD5+</th>
<th>CD56~CD5+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients*</td>
<td>121 ± 48</td>
<td>79 ± 34</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>Normals</td>
<td>19 ± 11</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

* Cell subsets were obtained from the peripheral blood of rIL-2-treated patients (n = 5) or normal donors (n = 3) by cell sorting and were cultured with 1500 units/ml rIL-2 for 3 days prior to the evaluation of cytolytic activity in 4-h chromium release assays.

Fig. 1. Flow cytometric contour plot depicting subsets of cells defined by expression of CD56 and CD16 cell surface markers in a patient undergoing rIL-2 therapy. Arrows, subsets.

Table 3 Acquisition of LAK activity by CD56~CD16+ and CD56~CD16- subsets from rIL-2-treated patients following in vitro activation with rIL-2

<table>
<thead>
<tr>
<th>Cell source</th>
<th>PBMCs</th>
<th>CD56~CD16+</th>
<th>CD56~CD16-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients*</td>
<td>124 ± 15</td>
<td>140 ± 90</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Normals</td>
<td>10 ± 2</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

* Cell subsets were collected, cultured, and assayed as described in Table 2.

Fig. 2. Comparison at limiting E:T ratios of the lytic activities of patient CD56+ cells (○), CD56~CD16+ cells (■), and PBMCs (□). Representative results from one of three independent experiments are shown.
CD56\(^{−}\) LAK PRECURSOR CELLS

The expansion of CD56\(^{+}\) cells during rIL-2 therapy has facilitated the functional and phenotypic characterization of the heterogeneous populations of non-MHC-restricted cytolytic cells which are present at frequencies too low for study in normal individuals. In the present report, we have identified a novel CD56\(^{−}\) NK cell/LAK precursor cell population in cancer patients receiving rIL-2 therapy. We and others have previously demonstrated that circulating LAK cells which develop during rIL-2 therapy are confined to subsets expressing the NK-associated marker CD56 (6, 12, 17). Despite the fact that circulating LAK effector cells are exclusively CD56\(^{+}\), we have observed that CD56\(^{−}\) cells from rIL-2-treated patients but not normal individuals exhibit NK-like activity and acquire LAK-like cytolytic activity following in vitro exposure to 1500 units/ml rIL-2 (13).

In the present studies we have confirmed the existence of a CD56\(^{−}\) LAK precursor cell in cancer patients undergoing rIL-2 therapy and have demonstrated that the cell exhibits a CD56\(^{−}\)CD16\(^{+}\)CD5\(^{+}\) phenotype. The existence of this subset was confirmed by extensive magnetic bead separation techniques to remove residual contaminating CD56\(^{+}\) LAK cells from fluorescence-activated cell sorter-purified populations. Although low levels of cytolytic activity were observed in CD56\(^{−}\)CD5\(^{+}\) cells in some experiments and the absolute number of LU recoverable in this population in some cases was comparable to the absolute LU in the less frequent CD56\(^{−}\)CD5\(^{−}\) fraction, the fact that activity in the CD56\(^{−}\)CD5\(^{+}\) subset was not consistently detected in all experiments leaves the significance of the cytolytic activity of this population open to question (Table 2). Additional observations argue against the possibility that the observed cytolytic activity is mediated by contaminating CD56\(^{+}\) cells or by CD16\(^{−}\)CD56\(^{+}\) granulocytes. CD56\(^{−}\) cells have never been detected in the sorted CD56\(^{−}\) populations, either prior to or following the in vitro culture period, even when two CD56\(^{−}\) epitope distinct monoclonal antibodies (anti-Leu 19, NKH-1) were combined for flow cytometric analysis (data not shown). Furthermore, if the observed CD16\(^{−}\)CD56\(^{−}\) cytolytic activity were due to contaminating CD56\(^{+}\) cells, one would also expect a similar contamination of other sorted CD56\(^{−}\) subsets (e.g., CD5\(^{+}\)), yet sorted CD56\(^{−}\)CD5\(^{+}\) cells failed to exhibit a similar level of cytolytic activity which would be expected if contamination by CD5\(^{+}\) cells occurred. Finally, the fact that purified CD56\(^{−}\)CD5\(^{+}\) cells exhibit lytic activity at E:T ratios too low for detection of lytic activity by IL-2-activated patient peripheral blood lymphocytes (comprising 15–25% CD5\(^{+}\) cells) indicates that the numbers of CD5\(^{+}\) cells required for detectable lytic activity are well above the minimal levels required for detection by flow cytometry. CD56\(^{−}\)CD16\(^{+}\) LAK precursors could also be distinguished from phenotypically related granulocytes based on (a) morphology, (b) higher levels of CD16 expression by granulocytes (18), or (c) higher levels of expression of LFA-1 on CD56\(^{−}\) LGLs.

The relationship between CD56\(^{−}\)CD16\(^{+}\) LAK cells and other cell types which mediate non-MHC-restricted cytotoxicity remains unclear. A majority of rIL-2-induced LAK cytotoxicity is mediated by NK cells bearing an LGL morphology and expressing the CD56 surface antigen, although a subpopulation of CD56\(^{+}\)CD3\(^{+}\) T-cells is also capable of mediating LAK activity (8, 12–14). More recent studies have described phenotypically unique subsets of NK-like precursor cells in the thymus during ontogeny. Phillips et al. (11) reported the existence of CD56\(^{−}\)CD16\(^{−}\) NK cells in human fetal cord blood, although

Table 6 Expression of cell surface antigens by CD56\(^{−}\)CD16\(^{+}\) LGLs

<table>
<thead>
<tr>
<th>Not expressed</th>
<th>CD3, CD4, CD8, CD14, CD19, CD25</th>
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<tbody>
<tr>
<td>CD3, CD4</td>
<td>CD11c (40%), CD16/Leu11c (30%)</td>
</tr>
<tr>
<td>CD8, CD14</td>
<td>CD11a, CD18, CD38</td>
</tr>
</tbody>
</table>

Table 7 Expansion of circulating CD16\(^{+}\)CD56\(^{+}\) cells during rIL-2 administration

<table>
<thead>
<tr>
<th>Mononuclear cell count (mm(^{3}) x 10(^{3}))</th>
<th>CD56(^{−})CD56(^{+})</th>
</tr>
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<tbody>
<tr>
<td>Pretherapy (n = 5)</td>
<td>1.0 ± 0.6(^{a})</td>
</tr>
<tr>
<td>Posttherapy (n = 5)</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Absolute cell count (mm(^{3}) x 10(^{3}))</td>
<td>75 ± 61</td>
</tr>
</tbody>
</table>

\(^{a}\) Cell counts were obtained before and 3 days after rIL-2 administration during the priming phase of rIL-2/LAK therapy.

\(^{b}\) Mean ± SEM.
cells bearing this phenotype were not detected in adult peripheral blood. Mingari et al. (10) reported the existence of a small subset of CD16^- cells in fresh human thymocyte suspensions which did not express other NK-associated markers, including CD56. These observations suggest the existence of immature forms of NK cells which express a phenotype similar to that of the LAK cell precursor described in the present report. It is therefore possible that administration of rIL-2 induces the differentiation, expansion, and/or peripheralization of CD56^- NK cells.

The observation that the CD56^- LAK cells described in the present studies do not express the T-cell-associated CD3, CD5, and TCR antigens indicates that this activity is not T-cell derived, although our studies do not exclude the possibility that LAK precursors exist in these populations but were not detectable under the conditions used. Although a majority of NK cells express CD56, the existence of a minor subpopulation of CD16^-CD56^-cells has been reported in low buoyant density fractions of human PBMCs that are highly enriched for NK cells (7). This subset was detected only in certain normal donors and comprised <5% of CD16^- NK cells. Although previous functional analyses of this subset have not been reported, our present studies demonstrate that this subset possesses NK activity in patients receiving rIL-2 and is capable of mediating non-MHC-restricted LAK cytolytic activity only after in vivo and in vitro exposure to rIL-2.

It is unclear why the CD56^-CD16^- cell subset requires previous in vivo exposure to rIL-2 before it is capable of rIL-2-induced activation in vitro. One possibility is that complete activation of CD56^-CD16^- cells to effector cell activity requires their exposure to high doses of rIL-2 plus other secondary cytokines. This cell subset might be exposed to adequate levels of cytokines during in vivo administration of rIL-2 but requires subsequent exposure to much higher doses of rIL-2, achievable only in vitro, for complete activation. Alternatively, complete activation of CD56^-CD16^- cells by rIL-2 in vitro might be inhibited by the production of secondary cytokines (such as IL-4) and only after exposure to rIL-2 in vitro does the functional activity of this subset become apparent (19). Both possibilities imply that in vivo exposure of these cells to rIL-2 and/or secondary cytokines is a necessary prerequisite for subsequent in vitro activation.

Clearly, the CD56^-CD16^- subset represents only a minor component of the circulating mononuclear cell pool and appears considerably less potent than its CD56^- counterpart. During rIL-2 therapy, this subset nevertheless undergoes limited expansion in patients receiving rIL-2, although their relative percentages remain low. Thus, despite the evidence for an rIL-2-induced effect on CD56^-CD16^- cells in vivo, these cells do not possess detectable rIL-2-induced LAK cytolytic activity when assessed immediately after isolation from patient blood.

The identification of functionally and phenotypically unique CD56^-CD16^- LAK precursor cells in patients receiving rIL-2 provides a new member of the heterogeneous family of cells capable of mediating LAK cytotoxicity. Further studies of this subset are required to elucidate its unique activation requirements and functional relationships to CD56^- LAK cells.

REFERENCES


Identification of a Novel CD56- Lymphokine-activated Killer Cell Precursor in Cancer Patients Receiving Recombinant Interleukin 2


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