Human Tumor Cell Line Resistance to Chemotherapeutic Agents Does Not Predict Resistance to Natural Killer or Lymphokine-activated Killer Cell-mediated Cytolysis

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ABSTRACT

Cancer cells selected for resistance to natural product chemotherapy agents typically display cross-resistance to a variety of structurally and mechanistically diverse agents, a phenomenon known as multidrug resistance. Preliminary studies involving cells selected for multidrug resistance in vitro have suggested that the development of resistance to these agents might simultaneously confer resistance to some forms of immunotherapy. Using human tumor cell line models, we have investigated the relationship between either intrinsic or selected multidrug resistance and sensitivity to natural killer (NK) or lymphokine-activated killer (LAK) cell-mediated cytolysis. We compared the NK and LAK cell susceptibilities of three human tumor cell lines displaying distinct mechanisms of selected drug resistance with that of the parental drug-sensitive lines. We also evaluated the NK and LAK susceptibility of five established renal cell carcinoma lines, all of which were found to be intrinsically resistant to doxorubicin and vinblastine. The drug-resistant cell lines were variably sensitive to NK-mediated lysis. In contrast, all drug-resistant cell lines tested were LAK cell sensitive. The NK and LAK cell-mediated cytolytic sensitivities of the drug-resistant cell lines correlated well with those of the drug-sensitive parental lines, suggesting that susceptibility to lysis was related intrinsically to each tumor type, and not to the resistance phenotype. We attempted to correlate the NK sensitivity of these cells with the cell surface expression of Class I or II histocompatibility antigens, or the presence or absence of the membrane inhibitor of complement-mediated reactive lysis. None of these phenotypic markers were found to predict NK resistance. We therefore conclude that these cells, which are either spontaneously resistant to commonly utilized antitumor agents or are multidrug resistant as a result of drug exposure in vitro, remain sensitive to LAK cell-mediated cytolysis. Our studies suggest that interleukin 2-induced LAK cells may be useful in the therapy of some chemotherapy-resistant cancers.

INTRODUCTION

Intrinsic and/or acquired resistance to cancer chemotherapeutic agents represent major obstacles to the successful treatment of human malignancies. A large percentage of commonly encountered solid tumors, such as lung, colon, prostate, and renal cell carcinomas, appears to be intrinsically resistant to currently available agents, while others, like breast cancer, appear to be initially sensitive to many different agents but ultimately become refractory to all of them. Clues to the cytokinetic and/or biochemical basis for this refractoriness to cancer chemotherapy have only recently emerged from the study of tumor cell line models (1). The in vitro selection of cell lines for resistance to natural product antitumor agents such as doxorubicin, daunorubicin, vincristine, and vinblastine has produced valuable models which have been instrumental in providing an understanding of the potential mechanisms of cellular drug resistance. Studies evaluating these models have demonstrated that the induction of resistance to one drug is frequently associated with the development of cross-resistance to a variety of structurally and mechanically unrelated agents (For review see Refs. 2 and 3). This phenomenon, termed MDR, is often accompanied by increased expression of a P, 170,000 plasma membrane glycoprotein, termed P-glycoprotein. Many of the natural product antineoplastic agents have been demonstrated to bind to P-glycoprotein and substantial evidence supports the hypothesis that the MDR phenomenon is mediated by enhanced cellular export of these chemotherapeutic agents by P-glycoprotein (4–6). High constitutive expression of P-glycoprotein is frequently seen in neoplasms derived from the gastrointestinal tract, liver, and kidneys and may account for the frequently observed chemotherapy resistance of tumors arising in these organs (7). Furthermore, the demonstration that cells from some human tumors which have become refractory to chemotherapy in vitro display evidence of increased P-glycoprotein expression (8–11) suggests that this phenotype may indeed play a role in cancer chemotherapy failure.

Recombinant DNA technology has enabled the production of a number of cytokines with potential antitumor activity. The efficacy of these agents against tumors refractory to conventional chemotherapy is currently being evaluated in clinical trials. One of these cytokines, IL-2, has the capacity to induce the proliferation of lymphocytes as well as the activation of tumoricidal lymphocytic activity (12). Lymphocytes which have been activated by IL-2, termed LAK cells, as well as circulating cytotoxic lymphocytes termed NK cells, have been demonstrated to play an important role in immune-mediated rejection of murine and human tumors (12–16). Patients with renal cell carcinomas and malignant melanomas, tumors which are typically refractory to therapy with standard chemotherapeutic agents, have recently been found to respond to the administration of either IL-2 alone or a combination of IL-2 plus LAK cells (17–19). Results of recent laboratory studies have suggested, however, that tumor cells selected for multidrug resistance in vitro may be refractory to NK cell-mediated killing, a process which is thought to be functionally similar to the cytotoxicity induced by LAK cells (20–22). In one such study, resistance to NK cell killing seemed to directly correlate with the level of tumor cell P-glycoprotein expression (20). In the limited number of studies reported to date, human tumor cells displaying MDR as a result of exposure to vincristine or doxorubicin have retained their sensitivity to LAK cells in vitro (23–25). In some of these models, however, details regarding

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3 The abbreviations used are: MDR, multiple drug or multidrug resistance; FBS, fetal bovine serum; NK, natural killer; LAK, lymphokine activated killer; MIRL, membrane inhibitor of reactive lysis; IL-2, interleukin 2; MTT, 3-[4,5-dimethylthiazol-2-yl] -2.5-diphenyltetrazolium bromide; IC50, the drug concentration which inhibits cell growth by 50%; HL-60/MX2, cloned HL-60 subline, ~35-fold resistant to mitoxantrone; MES-SA, human sarcoma cell line; MES-SA/MX, variant of MES-SA selected for resistance to mitoxantrone; α-MEM, α-modified Eagle’s medium; E:T ratio, effector:target cell ratio.
P-glycoprotein expression are not provided. Thus, the role that the development of multidrug resistance and/or P-glycoprotein expression plays in tumor cell killing by either NK or LAK effector cells remains unclear.

In this paper we demonstrate a lack of correlation between the development of human tumor cell multidrug resistance in vitro and susceptibility of those cells to either LAK- or NK-mediated tumor cell cytosis. Furthermore, in renal cell carcinomas with a wide range of "natural" drug sensitivities we found no correlation between the degree of cell line resistance to drug and the cytolytic efficacy of immune effector cells.

MATERIALS AND METHODS

Chemicals and Supplies. Doxorubicin was provided by Adria Laboratories (Columbus, OH). Vincristine and cisplatin were obtained from the Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD. Drugs were prepared by dissolving in dimethyl sulfoxide or 0.9% NaCl solution, with all dilutions made in 0.9% NaCl solution. Recombinant IL-2 was a gift from Cetus Corporation (Emeryville, CA). Rabbit antiserum to the MIRL (26) was generously provided by Dr. Charles Parker of the University of Utah School of Medicine. Monoclonal antibody C-219, which recognizes P-glycoprotein, was obtained from Centocor Laboratories (Malvern, PA). RPMI 1640, McCoy’s 5A, Waymouth’s, and α-MEM media; l-glutamine, penicillin; streptomycin; and fetal bovine serum were purchased from either GIBCO (Grand Island, NY) or Sterile Systems (Logan, UT).

Cells and Cell Culture. The human head and neck squamous cell carcinoma cell line SCC-25 and a variant selected for resistance to cisplatin (SCC-25/CP) (27) were kindly provided by Dr. Beverly Techer of the Dana Farber Cancer Institute, Boston, MA. SCC-25 and SCC-25/CP were maintained in continuous culture in α-MEM supplemented with 10% FBS, penicillin (100 IU/ml), streptomycin (100 µg/ml), and hydrocortisone (0.33 mg/ml). The HL-60 promyelocytic leukemia cell line and a cloned variant selected for resistance to the anthracycline compound mitoxanthrone (HL-60/MX2) (28) and the MES-SA sarcoma cell line (29) and a variant selected for resistance to mitoxanthrone (MES-SA/MX) have been reported from this laboratory. HL-60 and HL-60/MX2 were grown in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, and antibiotics. MES-SA and MES-SA/MX were grown in Waymouth’s:McCoy’s 5A medium (50:50) supplemented with 10% newborn calf serum and antibiotics. The human renal cell carcinoma lines CAKI-1, CAKI-2, and A-498 were obtained from the American Type Culture Collection and maintained in either McCoy’s 5A or Dulbecco’s modified Eagle’s medium supplemented with 10% FBS and antibiotics. Nonessential amino acids and sodium pyruvate were added to the A-498 medium. The RCC and RCSC renal cell carcinoma cell lines (30) were kind gifts from Dr. Daniel Terreros of the University of Utah School of Medicine and were grown in α-MEM containing 10% FBS and antibiotics. The NK-sensitive leukemia K-562 (American Type Culture Collection) and the LAK cell-sensitive, NK-resistant Daudi lymphoma cell lines (gift of A. Rayner, University of California, San Francisco, CA) served as positive controls. K-562 and Daudi were maintained in continuous culture by serial passage in Waymouth’s and McCoy’s 5A or Dulbecco’s modified Eagle’s medium supplemented with 10% FBS, penicillin (100 lU/ml), streptomycin (100 µg/ml), and 0.33 mg/ml. The HL-60 promyelocytic leukemia cell line and a cloned variant selected for resistance to the anthracycline compound mitoxanthrone (HL-60/MX2) (28) and the MES-SA sarcoma cell line (29) and a variant selected for resistance to mitoxanthrone (MES-SA/MX) have been reported from this laboratory.

Preparation of LAK Cell Suspensions. Peripheral blood lymphocytes were obtained by Ficoll-Hypaque gradient centrifugation of peripheral blood obtained from normal human volunteers. Following centrifugation at 1000 x g for 20 min, cells were resuspended in complete medium and either assayed directly for NK activity or resuspended in 75-cm² tissue culture flasks at 2 x 10⁶ cells/ml in X-vivo 10 medium (M. A. Bioproducts, Walkersville, MD) supplemented with 1000 units/ml of recombinant human IL-2. Following a 3–4-day culture period, the length of incubation shown by Lafreniere et al. (33) to result in optimal LAK cell induction, LAK cells were harvested by centrifugation.

Assays for Tumor Cell Killing by NK and LAK Cells. The cytotoxicity of IL-2-stimulated lymphocytes was assessed using a 4-h chromium release assay. Briefly, target cells derived from each tumor cell line were incubated for 60 min with 100 µCi ⁵¹Cr (Amersham, Arlington Heights, IL; specific activity, 250–500 mCi/mg)¹⁰¹⁰⁷ cells. Following extensive washes in X-vivo 10, a constant number of the labeled target cells (10⁵ cells/well) were added to varying numbers of unstimulated peripheral blood lymphocytes or LAK cells derived from normal donors in a microtiter plate, maintaining a constant volume of 200 µl/well. The maximum effector:target cell ratios in NK and LAK assays were 80:1 and 100:1, respectively. Control wells for measuring spontaneous chromium release contained 10⁴ target cells in 200 µl of medium. Total releasable chromium was determined by adding 100 µl of 1% Triton X-100 detergent (Bio-Rad, Richmond, CA) to 100 µl of radiolabeled target cells. The microtiter plate was centrifuged briefly at 250 x g and then incubated for 4 h in a humidified 5% CO₂ incubator at 37°C. Chromium release was quantified by centrifuging microtiter plates at 500 x g for 5 min and harvesting 100 µl of supernatant from each well for gamma counting. Specific cytotoxicity was calculated from the average of triplicate wells as

\[
\text{Specific cytotoxicity} = \frac{\text{Experimental cpm} - \text{spontaneous release cpm} \times 100}{\text{Total release cpm} - \text{spontaneous release cpm}}
\]

Cell line sensitivity to LAK or NK cell killing was defined as ≥15% specific cytotoxicity at effectortarget cell ratios of 100:1 and 80:1, respectively.

The in Vivo and Indirect Immunofluorescence Assays. Cells derived from each of the adherent tumor cell lines were dissociated with 1% collagenase (Sigma)/1% dispase (Boehringer-Mannheim, Indianapolis, IN) to minimize proteolytic cleavage of cell surface molecules. Both these and nonadherent cell lines (HL-60, Daudi, K-562) were extensively washed in serum free medium and then resuspended at 10⁶ cells/ml in ice cold phosphate-buffered saline with 1% bovine albumin and 0.1 M sodium azide (modified phosphate-buffered saline). The suspended cells were then pelleted and each pellet was incubated at 4°C for 30 min with a predetermined optimal amount of monoclonal antibody to Class I monomorphic determinants (HLA-ABC.1, an unlabeled mouse anti-human IgG3a; Accurate Chemical Corporation, Westbury, NY), or anti-HLA-DR monoclonal antibody (HLA-DR3, phycoerythrin-labeled mouse anti-human IgG2a, Becton Dickinson, Sunnyvale, CA). Tumor cells were also stained with a rabbit antisera that recognizes and

* W. G. Harker and D. L. Slade, manuscript in preparation.
blocks the functional activity of the MIRL (26). Staining was followed by extensive washes in ice cold modified phosphate-buffered saline. Samples requiring analysis by indirect immunofluorescence were subsequently stained with a phycoerythrin-conjugated secondary goat anti-mouse IgG antiserum (preadsorbed with human and rat IgG), or a fluorescein-conjugated goat anti-rabbit IgG antiserum. Following repeated washes, cells were fixed in 1% paraformaldehyde and analyzed by fluorescence-activated cell sorter. Unstained cells, or cells stained with secondary antibody alone, served as controls for fluorescence-activated cell sorter analysis. The fraction of fluorescence-labeled cells was established by the computer-aided subtraction of histograms generated from the analysis of 10^6 labeled cells from histograms generated from unstained cells (direct immunofluorescence assay), or cells stained with the secondary antibody alone (indirect immunofluorescence assay).

**Results**

**In Vitro Drug Sensitivity.** The susceptibility of the various human carcinoma and leukemia cell lines to the chemotherapeutic agents vincristine, doxorubicin, and cisplatin is shown in Table 1. The MTT-defined doxorubicin IC50 for these lines ranged from a low of 80 ng/ml for the MES-SA sarcoma cell line to a high of >100 μg/ml for the CAKI-2 renal carcinoma lines. The vinblastine IC50 for these cells ranged from 7 ng/ml for the SCC-25 squamous cell carcinoma line to >100 μg/ml for CAKI-1, CAKI-2, and the RCC and RCSC renal carcinoma lines. The HL-60 leukemia cells were most sensitive to cisplatin (IC50 230 ng/ml) and the RCC, CAKI-1, and CAKI-2 cells least sensitive (IC50 >100 μg/ml). The HL-60/MX2 and MES-SA/MX lines, both of which were selected for resistance to mitoxantrone, displayed marked cross-resistance to amsacrine, etoposide, teniposide, daunorubicin, doxorubicin, and bisantrene compared to their respective parental lines (28). The HL-60/MX2 line, however, retained sensitivity to the Vinca alkaloids vincristine and vinblastine as well as to mitomycin C and melphanal, whereas the MES-SA/MX cells displayed varying degrees of cross-resistance to these agents as well. The SCC-25/CP cells, which were selected for resistance to cisplatin, have been reported to be 30-fold less sensitive to cisplatin than the SCC-25 parent (27) but remained as sensitive to vinblastine and doxorubicin as the SCC-25 cells.

**Susceptibility to NK Cell- and LAK Cell-mediated Cytotoxicity.** The susceptibility of the five renal cell carcinoma cell lines and the paired sensitive and resistant cell lines, HL-60 and HL-60/MX2, MES-SA and MES-SA/MX, and SCC-25 and SCC-25/CP, to NK cell-mediated lysis, was evaluated by 4-h 51Cr release assay. The specific lysis of the paired cell lines, at 4 different E:T ratios, is shown in Fig. 1A. Both drug-sensitive and -resistant pairs of HL-60 and SCC-25 cell lines were resistant to NK cytotoxicity, with <10% specific lysis noted for each cell type. The mitoxantrone-sensitive (P-glycoprotein-negative) 51Cr release assay. Peripheral blood lymphocytes from normal donors were separated by Ficoll-Hypaque centrifugation and tested directly for NK lytic activity against the tumor cells, using a 4-h 51Cr release assay. Peripherale lymphocytes from the same donor source were assayed for LAK activity against these cell lines following a 3–4-day incubation with IL-2 (6000 IU/ml) in vitro. Cell lines: O, MES-SA; ●, MES-SA/MX; △, HL-60; ▲, HL-60/MX2; ◆, SCC-25; ■, SCC-25/CP. The NK-sensitive leukemia K-562 cells (x) and LAK-sensitive Daudi lymphoma cells (●) served as controls for the studies depicted in A and B, respectively. Each point represents the mean of at least 4 determinations.

**Table 1 Drug sensitivity of human tumor lines**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Doxorubicin (μg/ml)</th>
<th>Vinblastine (μg/ml)</th>
<th>Cisplatin (μg/ml)</th>
<th>P-glycoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC-25</td>
<td>0.20</td>
<td>0.007</td>
<td>NT</td>
<td>—</td>
</tr>
<tr>
<td>SCC-25/CP</td>
<td>0.24</td>
<td>0.008</td>
<td>NT</td>
<td>—</td>
</tr>
<tr>
<td>HL-60</td>
<td>0.08</td>
<td>0.008</td>
<td>0.23</td>
<td>—</td>
</tr>
<tr>
<td>HL-60/MX2</td>
<td>0.32</td>
<td>0.008</td>
<td>0.29</td>
<td>—</td>
</tr>
<tr>
<td>MES-SA</td>
<td>0.1</td>
<td>0.01</td>
<td>1.25</td>
<td>—</td>
</tr>
<tr>
<td>MES-SA/MX</td>
<td>19.9</td>
<td>0.94</td>
<td>1.80</td>
<td>+</td>
</tr>
<tr>
<td>A-498</td>
<td>1.0</td>
<td>64.8</td>
<td>5.2</td>
<td>—</td>
</tr>
<tr>
<td>RCC</td>
<td>46.6</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>—</td>
</tr>
<tr>
<td>RCSc</td>
<td>72.0</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>—</td>
</tr>
<tr>
<td>CAKI-1</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>—</td>
</tr>
<tr>
<td>CAKI-2</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>—</td>
</tr>
</tbody>
</table>

* Concentration of chemotherapeutic agent inhibiting cell growth by 50% as measured by the MTT assay.

* P-glycoprotein expression as determined by immunohistochemical staining.

* NT, not tested; the SCC-25/CP line is reported to be 30-fold less sensitive to cisplatin than the SCC-25 parental line (27).
carcinoma lines, which displayed intrinsic resistance to doxorubicin, vinblastine, and cisplatin, are shown. The A498, CAKI-1, and CAKI-2 cell lines displayed borderline sensitivity to NK cytolysis (specific lysis, ~15% at an E:T ratio of 80:1) while the RCC and RCSC cell lines were resistant (specific lysis, 1 and 7.5%, respectively).

Despite the varied NK and drug susceptibilities of the paired sensitive and resistant cell types and the 5 renal carcinoma lines, each of the 11 cell lines examined was demonstrated to be sensitive to LAK cell-mediated killing (Figs. 1B and 2B). As expected there was variability in the sensitivity of these cell lines to LAK cell killing but there was no correlation between the LAK cell susceptibility of the cell lines and their in vitro sensitivity to any of the three chemotherapeutic agents tested.

Cell Surface Marker Expression. Mulé et al. reported that Class I major histocompatibility antigen expression by murine tumors appeared to be predictive of their sensitivity to NK and LAK cell killing (36). We therefore attempted to correlate Class I or II antigen expression with NK cell sensitivity in our human cell lines (Table 2). These experiments failed to demonstrate a correlation between NK or LAK resistance and Class I or Class II antigen expression. In particular, the MES-SA and CAKI-1 cell lines expressed no demonstrable Class I antigen yet were sensitive to NK-mediated cytolysis. Furthermore, all of these cell lines were susceptible to LAK-mediated cytolysis, regardless of the level of Class I antigen expression. Only the Daudi lymphoma cells (NK resistant, LAK sensitive) expressed Class II antigens.

Since cytotoxic cells produce pore-forming proteins (perforins) that damage the membranes of target cells, similar to the membrane attack complex of complement (37), we sought to determine whether the cell surface expression of the recently described M, 18,000 MIRL correlated with the susceptibility of tumor cells to NK or LAK cell killing. All of the tumor cell lines (both NK and LAK sensitive) stained positively for MIRL. Thus the cytotoxicity of NK or LAK cells for these human tumor cell lines did not correlate with the presence or absence of cell surface MIRL expression.

P-Glycoprotein Expression. Both the colchicine-resistant murine CHF8C5 cells and the mitoxantrone-resistant human MES-SA/MX cells stained positively for P-glycoprotein with the C-219 monoclonal antibody. Neither of the drug-sensitive counterparts, however, AUXB1 or MES-SA, respectively, stained with the C-219 antibody. Nor was there C-219 staining of cells from either the mitoxantrone-sensitive or -resistant HL-60 leukemia lines. Surprisingly, the five renal carcinoma cell lines

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**Table 2 Analysis of tumor cell surface markers that may predict sensitivity to immunological recognition**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>HLA-ABC</th>
<th>HLA-DR</th>
<th>MIRL</th>
<th>NK lysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC-25</td>
<td></td>
<td></td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>SC-25/CP</td>
<td></td>
<td></td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>HL-60</td>
<td></td>
<td>81</td>
<td>0</td>
<td>R</td>
</tr>
<tr>
<td>HL-60/MX</td>
<td>71</td>
<td>0</td>
<td>72</td>
<td>R</td>
</tr>
<tr>
<td>MES-SA</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>S</td>
</tr>
<tr>
<td>MES-SA/MX</td>
<td>0</td>
<td>0</td>
<td>96</td>
<td>S</td>
</tr>
<tr>
<td>A-498</td>
<td>70.2</td>
<td>0</td>
<td>88</td>
<td>S</td>
</tr>
<tr>
<td>Caki-1</td>
<td>1.3</td>
<td>0</td>
<td>+</td>
<td>S</td>
</tr>
<tr>
<td>Caki-2</td>
<td>58.1</td>
<td>0</td>
<td>21</td>
<td>S</td>
</tr>
<tr>
<td>RCC</td>
<td>51.1</td>
<td>0</td>
<td>50</td>
<td>R</td>
</tr>
<tr>
<td>RCSC</td>
<td>5.5</td>
<td>0</td>
<td>42</td>
<td>R</td>
</tr>
<tr>
<td>K-562</td>
<td>53</td>
<td>0</td>
<td>23</td>
<td>S</td>
</tr>
<tr>
<td>Daudi</td>
<td>0</td>
<td>78.8</td>
<td>80</td>
<td>R</td>
</tr>
<tr>
<td>NL PBL</td>
<td>61.4</td>
<td>61.7</td>
<td>73</td>
<td>–</td>
</tr>
</tbody>
</table>

* Tumor cells and normal peripheral blood lymphocytes (positive control) were stained for HLA-ABC or MIRL expression by indirect immunofluorescence and for HLA-DR expression by direct fluorescence. Staining of the tumor cells was analyzed by fluorescence-activated cell sorter analysis of 10⁴ stained cells.

a Results are expressed as the percentage of analyzed cells staining positively on fluorescence-activated cell sorter analysis and represent the average of at least two determinations. * indicates that many of the cells were found to stain positively with that particular antibody but the analysis was only performed once.

b R, NK-resistant (<15% specific cytotoxicity in ⁵¹Cr release assay, at 80:1 effector:target cell ratio). S, NK-sensitive (≥15% specific cytotoxicity in ⁵¹Cr release assay).
were also P-glycoprotein negative by immunohistochemical staining with the C-219 antibody, despite their marked resistance to both vinblastine and doxorubicin and the high constitutive levels of P-glycoprotein expressed in normal renal tissues (38) and other renal carcinoma cell lines (39).

DISCUSSION

The emergence and outgrowth of drug-resistant tumor cells during the course of cancer chemotherapy usually denote treatment failure and ultimate patient demise. Recently, adoptive immunotherapy with in vitro-activated lymphocytes plus IL-2 has emerged as an effective treatment for some patients with renal cell carcinomas and malignant melanomas, tumor types which typically display intrinsic resistance to conventional cancer chemotherapy (17–19). In addition, animal studies have suggested that the combination of IL-2/LAK plus chemotherapeutic agents might have synergistic antitumor effects (36). The hope that these studies raise for a curative adoptive immunotherapy regimen for tumors with acquired or intrinsic drug resistance must be tempered by the results of recent studies which suggest that the emergence of multidrug-resistant cell populations during drug treatment might somehow simultaneously lead to tumor cell resistance to NK cell killing (20, 21). The latter studies prompted our further examination of the role that the MDR phenotype plays in defining human tumor cell susceptibility to adoptive immunotherapy.

We have investigated the in vitro cytotoxic activity of two host immune effectors, NK cells and LAK cells, against human tumor cell lines with either intrinsic or acquired resistance to chemotherapeutic agents. For these studies we chose tumor cell line models which were representative of both P-glycoprotein-positive and -negative MDR, also called classic and atypical MDR (40), as well as models of resistance to cisplatin, an agent thought to act via DNA alkylation and not known to lead to the development of either the classic or atypical MDR phenotype. Our results indicate that the NK cell susceptibility of tumor cells which have been selected for resistance to antineoplastic agents in vitro is quite variable and does not correlate well with the degree of in vitro sensitivity to doxorubicin, vinblastine, or cisplatin. Thus, HL-60 leukemia cells selected for resistance to mitoxantrone and SCC-25 squamous carcinoma cells selected for resistance to cisplatin, both of which displayed resistance to multiple drugs in the absence of P-glycoprotein expression, retained the same level of NK cell sensitivity/resistance displayed by their drug-sensitive parental counterpart. Similarly, NK-sensitive MES-SA sarcoma cells, and the MES-SA/MX subline which displayed P-glycoprotein-mediated MDR following selection in mitoxantrone, displayed equal sensitivity to NK cell-mediated cytosis.

Previous studies in which the NK or LAK susceptibility of drug-resistant murine or human cell lines has been examined have provided conflicting results. In most of the paired drug-sensitive/resistant models examined to date the NK susceptibilities of the resistant subline were identical to that of the sensitive parental cell type (22, 23). Sugimoto et al. (22), however, reported that the NK susceptibility of L5178Y murine leukemia cells, which had been selected for resistance to aclacinomycin, was increased compared to drug-sensitive parental controls. In contrast, Woods et al. (20) noted that NK-sensitive murine A20-HL and YAC-1.2 lymphoma cells, which were selected for resistance to either doxorubicin or vincristine in vitro, became less sensitive than the parental cells to NK cytolysis. The E:T ratios utilized in these experiments were quite low (8:1), however, and each of their lines would have been considered NK sensitive by our criteria. A similar decrease in sensitivity to NK cell-mediated cytolysis was also reported in a multidrug-resistant K562 erythroleukemia cell line selected for resistance to daunorubicin (21). A 20–60% inhibition of NK lysis was noted in the resistant cells at E:T ratios varying from 3:1 to 30:1. While the etiology for the reduced NK susceptibility of these cells could not be determined, it did not appear to occur as a result of altered effector:target binding.

We subsequently evaluated the LAK susceptibilities of our drug-sensitive and -resistant cell line pairs as well as the renal cell lines. All eight drug-resistant lines and the three drug-sensitive parental lines (HL-60, MES-SA, and SCC-25) were sensitive to LAK killing in vitro. The LAK cell sensitivities of 8 other drug-resistant cancer cell lines (4 murine, 4 human), which had been selected for resistance to a variety of antineoplastic agents, have been reported previously (23, 24, 41). In each case the cells were found to be sensitive to LAK cell-mediated killing, confirming our observations. These findings highlight one obvious discrepancy between the observations made in these in vitro studies and the results of the few human clinical LAK/IL-2 trials reported to date; i.e., despite the nearly universal sensitivity of fresh tumor cells and cell lines (including MDR cells) to LAK cytolysis in vitro, only a minority of patients who receive IL-2/LAK therapy will respond (17). Furthermore, a controversy exists in the literature as to whether NK- and LAK-induced cytotoxicity are mediated by the same or different cell types. The current studies, while suggestive that the cytotoxicities might be mediated by different cell types, were not specifically designed to address that question. Clearly, additional studies are needed to determine the factor(s) responsible for the resistance of these tumors to LAK/IL-2 administration in vivo and to determine the cell type(s) responsible for the tumor regressions noted clinically.

In an attempt to find phenotypic correlates of NK sensitivity or resistance, we examined Class I or Class II antigen expression by tumor cells. In the cell lines examined, there was no correlation between Class I expression and NK or LAK susceptibility. As expected, too few cell lines expressed Class II antigen for a meaningful analysis. We further attempted to correlate tumor cell expression of MIRL, a possible inhibitor of complement-like proteins contained within LAK cell granules, with resistance to NK-mediated lysis. MIRL was expressed in all of the NK-sensitive and -resistant tumor cells studied and expression therefore was not predictive of immune effector resistance.

Our current studies indicate that neither intrinsic tumor cell resistance to certain cytotoxic agents nor the emergence of a multidrug-resistant population following exposure to these agents necessarily predicts for subsequent NK or LAK cell susceptibility. They further suggest that chemotherapy and IL-2-based immunotherapy may be non-cross-resistant forms of cancer therapy. Studies designed to evaluate the efficacy of IL-2/LAK therapy in patients following chemotherapy failure have recently been initiated.

REFERENCES

Human Tumor Cell Line Resistance to Chemotherapeutic Agents Does Not Predict Resistance to Natural Killer or Lymphokine-activated Killer Cell-mediated Cytolysis

W. Graydon Harker, Corrynne Tom, John R. McGregor, et al.


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