Susceptibility to LAK-mediated Cytotoxicity of Multidrug-resistant Variants of the Human RAJI Cell Line is Not Related to Expression of Major Cellular Adhesion Molecules[1]

Robin S. Treichel, Department of Biology, Oberlin College, Oberlin, OH 44074-1082

ABSTRACT. The association of multidrug resistance (MDR) with resistance to lysis by natural killer (NK) and lymphokine-activated killer (LAK) cells is controversial. To address this issue further, drug-resistant variants of a human Burkitt lymphoma cell line (RAJI) were developed in vitro by intermittent exposure to Adriamycin® (R/ADR) or to etoposide (R/VP-16). The RAJI cell line was selected because it is a standard LAK-susceptible target. Both MDR lines as well as the parent cell line were found to be resistant to NK-mediated lysis, but highly susceptible to LAK-mediated lysis. Notably, R/ADR cells, which express high levels of P-glycoprotein (P-gp), were nearly eight-times more susceptible to LAK-mediated lysis than parental cells, whereas R/VP-16 cells, which are P-gp-negative, were equally as susceptible as parental cells. Immunofluorescence analyses revealed that expression of cellular adhesion molecules that have been reported to control susceptibility of targets to NK- and LAK-lysis (ICAM-1, LFA-1, LFA-3, and MHC class I) did not differ significantly between the RAJI parent line and drug-resistant variants. This finding suggests that the increased LAK-sensitivity of R/ADR results from alterations which affect post-binding stages of the LAK lytic pathway.

INTRODUCTION

The development of resistance to anticancer agents by tumor cells is a common and serious clinical problem. In vitro selection of cell lines with natural product agents such as doxorubicin (Adriamycin®, ADR), vincristine or etoposide (VP-16) has produced sublines which are resistant to a variety of structurally and functionally unrelated agents (Curt et al. 1984, Danks et al. 1987). This phenotype is termed multidrug-resistance (MDR) and approximates that seen clinically (Curt et al. 1984).

Frequently, MDR is associated with increased expression of a M_r 170,000 plasma protein, termed the P-glycoprotein (P-gp), which apparently functions as an efflux pump to reduce the intracellular accumulation of natural product agents. P-gp-associated MDR has been demonstrated clinically in several malignancies, including acute leukemia (Fojo 1989, Kuwazuru 1990, Mattern et al. 1989). Another form of MDR is associated with an alteration in DNA topoisomerase II activity, resulting in fewer drug-induced breaks of DNA strands (Glisson et al. 1986). Cells with this "atypical" form of MDR (Danks et al., 1987) are resistant to anthracyclines and epipodophyllotoxins, but in contrast to cells with P-gp-associated MDR, retain susceptibility to *Vinca* alkaloids, do not over-express P-gp, and do not exhibit reduced intracellular drug accumulation. Cells selected for ADR-resistance may have additional mechanisms which contribute to their MDR phenotype including reduced drug uptake, enhanced detoxification by glutathione-S-transferases (Singh et al. 1989) and early DNA repair (Baas et al. 1990, Deffie et al. 1988).

Adoptive immunotherapy with interleukin-2 (IL-2)-activated lymphocytes (LAK) has been proposed as an approach to overcome MDR (Adler et al. 1988, Higuchi et al. 1989, Lotzova et al. 1990). Activity of LAK cells has been shown to be mediated primarily by natural-killer (NK) cells (Lotzova et al. 1990). Although NK cells are capable of spontaneously killing a variety of tumor cells, activation with IL-2 enhances their activity and increases the range of susceptible tumor targets (Grimm et al. 1982). An important question is whether tumor cells which exhibit MDR remain susceptible to lysis by LAK cells. There have been several recent studies to address this problem (Harker et al. 1990, Kimmig et al. 1990, Ohtsu et al. 1989, Scheper et al. 1991), but the studies have been limited in number and have yielded conflicting results.

To further examine the possible relationship between MDR and LAK susceptibility, we developed two MDR sublines of the human Burkitt lymphoma cell line RAJI. The RAJI cell line was selected because it is a standard target used for the study of LAK activity. Our parental and drug-resistant lines were compared for susceptibility to LAK lysis. Because increased expression of P-gp has been associated with altered cell-surface expression of the cellular adhesion molecules which play a role in NK/LAK target-cell interactions (Robertson et al. 1990, Scheper et al. 1991), we also examined the expression of major adhesion molecules on our parent and drug-resistant cells.

MATERIALS AND METHODS

Chemicals and Supplies

RPMI 1640 media with and without phenol red were purchased from GIBCO (Grand Island, NY); fetal bovine serum was from Flow Laboratories (McLean, VA) and from Hyclone Laboratories (Logan, UT). Etoposide (VP-16, VePesid®) was generously supplied by Bristol-Myers (Evansville, IN) and ADR was generously supplied by Adria Laboratories (Dublin, OH); vincristine (Oncovin®)

was purchased from Lilly (Indianapolis, IN). The tetrazolium salt 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]2H-tetrazolium hydroxide was obtained from Polysciences, Inc. (Warrington, PA). Phenazine methosulfate, triton-X, penicillin, streptomycin, ficoll-hypaque and IL-2 were obtained from Sigma (St. Louis, MO). Gammascint™ scintillation fluid was obtained from National Diagnostics (Manville, NJ). The C219 monoclonal antibody was purchased from Centocor (Malvern, PA); DAKO-CD11a, DAKO-CD18, DAKO-CD56, and DAKO-HLA-A,B,C were purchased from DAKO (Carpinteria, CA); CT-CD29 and CT-CD54 from Biosource International (Camarillo, CA); AI-CD58 from AMAC, Inc. (Westbrook, ME); I3 from Coulter (Hialeah, FL); and the murine IgG2a myeloma protein (UPC 10) from Organon Teknika (Durham, NC). Fluorescein-conjugated goat anti-mouse IgG and bovine serum albumin (Cohn fraction V) were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN).

Cell Lines

The Burkitt lymphoma cell line RAJI was obtained from the American Type Culture Collection (CCL 86, Rockville, MD). The MDR sublines CEM/VLB$_{100}$ and CEM/VM-1 (Beck et al. 1987, Danks et al. 1987) were provided by Dr. W. T. Beck, St. Jude Children's Research Hospital (Memphis, TN). Cells were grown as a suspension culture in RPMI 1640 medium supplemented with 10% FBS; penicillin, 50 IU/ml; streptomycin, 50 µg/ml; and for CEM variants, 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO$_2$ in air and sub-cultured every two days. Routine testing for *Mycoplasma* contamination proved negative.

The R/ADR subline was derived by culturing parent cells in 10 mM ADR and gradually increasing the concentration to 60 nM over a period of 10 months. The R/VP-16 subline was derived over a period of 6 months by increasing VP-16 from 10 nM to a final concentration of 400 nM. Growth rates and viabilities of the resistant sublines are equivalent to those of the parent line.

A large number of ampoules of the resistant cultures were frozen. In order to prevent genetic drift, cells were maintained in culture for no more than two months and then were replaced with freshly thawed cultures. Thawed drug-resistant cells were cultured with selecting drug for four to seven days, but were always maintained without drug for at least one week prior to assay.

Drug Sensitivity Studies

The sensitivities of the drug-resistant and parent cell lines to ADR, vincristine, and VP-16 were compared using a modification of a soluble tetrazolium/formazan assay (Scudiero et al. 1988). Logarithmically growing cells were washed, resuspended in RPMI 1640 without phenol red supplemented with 10% FBS and antibiotics and incubated at a final concentration of 2.5 x 10^5/ml with drug in a total volume of 200 µl in wells of 96-well flat-bottom microplates. Anti-cancer compounds were diluted in 0.9% saline prior to addition to wells. After three days, 100 µl of a freshly prepared solution of phenazine methosulfate (0.03 mM) in

XTT (1 mg/ml in serum-free medium) was added per well and incubation continued for 3-5 hours. Plates were then shaken and the absorbance measured at 450 nm with a Vmax microplate reader (Molecular Devices, Menlo Park, CA). Assay points were determined from six replicate wells and experiments were repeated at least three times. Wells containing phenazine methosulfate-XTT in medium without cells served as the plate blank. Parent and drug-resistant lines were always tested in parallel for sensitivity to a particular compound. The drug concentration was determined which reduced by 50% (IC$_{50}$) the A$_{450}$ in wells containing drug as compared to control wells without drug. Relative resistance was calculated by dividing the IC$_{50}$ of the resistant line by the IC$_{50}$ of the parent line.

Immunofluorescence Analysis

The C219 monoclonal antibody was used to detect a conserved epitope of the cytoplasmic domain of P-gp (Kartner et al. 1985). Drug-resistant cells, parental cells, and cell mixtures were evaluated by indirect immunofluorescence using a slight modification of the previously described procedure (Kartner et al. 1985). Fixed cells (10^6) were incubated overnight at 4°C with 1 µg C219, washed twice and then incubated for one hour at 4°C with FITC-anti-mouse IgG diluted 1/60. Washed cells were then resuspended in freshly prepared 50% glycerol in PBS containing 20 mg/ml 1,4-diazobicyclo-(2,2,2)octane and examined on a Nikon epifluorescence microscope. Background staining, determined using the isotype-matched murine myeloma protein UPC 10 as primary antibody, was uniformly weak with all lines. To calibrate the staining reactions, the P-gp-positive CEM/VLB$_{100}$ and P-gp-negative CEM/VM-1 cells which exhibit known degrees of P-gp expression (Beck et al. 1987) were simultaneously stained with C219 and isotype antibody. Experiments were performed twice with identical results.

Expression of adhesion molecules was determined using a standard indirect immunofluorescence assay (Stong et al. 1985) with the anti-ICAM-1 antibody CT-CD54, the anti-LFA-1 antibodies DAKO-CD11a and DAKO-CD18, the anti-LFA-3 antibody AI-CD58, the anti-human integrin ß-1 chain antibody CT-CD29, the anti-human natural killer cell antibody DAKO-CD56 as well as the anti-MHC class I and class II framework antibodies DAKO-HLA-A,B,C and I3. Monoclonal antibodies were used at concentrations recommended by the manufacturers. At least 100 cells were evaluated for each sample; fluorescence intensity was subjectively graded on a scale from weak (+) to very strong (++++). Experiments were performed twice with similar results.

NK and LAK Cytotoxicity Assays

Drug-resistant variants were cultured in drug-free medium for at least two weeks prior to use as targets in a standard ^{51}Cr-release assay. Mononuclear cells from peripheral blood of healthy adult volunteers were isolated by ficoll-hypaque gradient centrifugation, and plastic nonadherent cells were used directly as effectors (NK) or stimulated for five days with 100 units/ml of recombinant human IL-2 (LAK effectors). Stimulated effector cells were

washed before use. Varying numbers of effector cells were incubated in triplicate with 10,000 or 20,000 ^{51}Cr-labeled targets in 200 µl in microtiter plates for four hours at 37°C in a humidified atmosphere of 5% CO_2 in air. Plates were then centrifuged (500 x g, 10 min) and 100 µl of supernatant from each well was placed in a vial with 3 ml of scintillation fluid and radioactivity determined with a Beckman scintillation counter. Percent specific cytotoxicity was calculated as follows:

% specific lysis =

$$\frac{\text{test cpm - spontaneous cpm}}{\text{total cpm - spontaneous cpm}} \times 100\%.$$

Spontaneous ^{51}Cr release was determined by incubating target cells in medium without effectors. Total counts were determined by incubating target cells in 1% Triton-X; total cpm ranged from 7,600-48,900 depending on the target line and number of cells plated. Spontaneous release was always less than 10% of total ^{51}Cr release. One lytic unit was defined as the number of effector cells required to lyse 40% of 10,000 targets; the number of lytic units per 10^8 effectors ($LU_{40}/10^8$) was calculated. Drug-resistant and drug-sensitive cell line pairs were always tested in parallel within the same experiment. Three experiments using effectors from different donors yielded results which were essentially identical. The statistical significance of differences between drug-resistant sublines and the parent cell line was determined by Student's t test for paired samples.

RESULTS

Cross-resistance to Anti-cancer Agents

Parent and drug-resistant cell lines were evaluated for sensitivity to several anti-cancer agents encompassed within the spectrum of MDR (Table 1). The R/ADR and R/VP-16 lines were 7.4-fold and 52.1-fold, respectively, more resistant than RAJI to their selecting agents. Each subline also exhibited resistance to additional drugs. R/ADR exhibited low-level resistance to vincristine (3.2-fold) and surprisingly was more resistant to VP-16 (22-fold) than to the selecting agent ADR. The R/VP16 line, in contrast, exhibited cross-resistance to ADR (5.9-fold) but not to vincristine (1.1-fold).

TABLE 1

Drug sensitivity of drug-resistant sublines derived from the human Burkitt lymphoma line RAJI.

| Cell Line | IC_{50} (nM)[a] | | |
	Adriamycin	Vincristine	Etoposide
RAJI	48.6 ± 2.6	1.68 ± 0.11	179 ± 21
R/ADR	357.7 ± 12.3	5.31 ± 0.37	3947 ± 587
R/VP-16	284.7 ± 12.5	1.91 ± 0.16	9325 ± 1563

[a]The IC_{50} is the concentration of chemotherapeutic agent that inhibits cell growth by 50% in the XTT colormetric assay. Lymphoma cells in six replicate wells at 2.5 x 10^4 cells/well were incubated with 8-10 concentrations of anti-cancer agent for three days at 37°C. XTT with phenazine methosulfate was added and incubation continued for 3-5 hours followed by determination of the A_{450}. Parent and variant sublines were always tested in parallel. Values represent the mean ± s.d. of at least three independent experiments.

P-Glycoprotein Expression

The P-gp-specific C219 monoclonal antibody stained RAJI cells more intensely than did the control isotypic antibody and produced a fluorescence intensity which was slightly but reproducibly stronger than that observed with P-gp-negative CEM/VM-1 cells. R/ADR reacted strongly with C219; stained cells exhibited a fluorescence intensity which was much greater than that of C219-stained RAJI cells and nearly as bright as that of identically treated CEM/VLB_{100} cells. In contrast, R/VP-16 stained no more intensely with C219 than with control isotype antibody. These results indicate that R/VP-16 is negative, RAJI is weakly positive and R/ADR is strongly positive for P-gp expression.

Susceptibility to NK- and LAK-mediated Cytotoxicity

As expected, the drug-resistant and parental cells were resistant to NK cell-mediated cytotoxicity (Fig. 1); less than 10% specific lysis was observed in a 4-hr ^{51}Cr-release assay at even the highest effector:target ratios. Stimulation of effectors with IL-2 for 5 days markedly increased lytic activity against all three targets. At an effector:target ratio of 40:1 all targets showed at least 50% lysis. Notably, R/ADR was markedly more susceptible than the parent line with effectors from all donors tested ($P < 0.001$). Comparison of activity expressed as lytic units indicates that R/ADR ($LU_{40}/10^8$ = 262,467) was 7- to 8-times more susceptible to LAK-lysis than RAJI ($LU_{40}/10^8$ = 33,367) or R/VP-16 ($LU_{40}/10^8$ = 37,037).

Cell Surface Expression of Adhesion Molecules

No marked differences in expression of adhesion molecules were detected between parental and resistant cell lines. CD29 expression varied somewhat with moderate expression (++) by RAJI and R/ADR and low-level expression (+) by R/VP-16. For the other antigens assayed, no differences between parent and resistant cells were observed in percent positive cells or in fluorescence intensity. All cell lines strongly expressed MHC class I (++++), MHC class II (++++), and ICAM-1 (+++) molecules. Cells also expressed low levels of LFA-3 (+) and LFA-1 (+), but were negative for the human NK cell antigen CD56.

DISCUSSION

Experimental data suggest that adoptive immunotherapy with IL-2/LAK cells may be a useful treatment for leukemia (Adler et al. 1988, Higuchi et al. 1989, Lotzova et al. 1990). Given the proven efficacy of chemotherapy, most patients who become candidates for immunotherapy will have failed previous chemotherapeutic regimens. In these patients, immune effector cells will likely encounter tumor populations which have survived and therefore may be resistant to the cytotoxic effect of drugs. Several recent reports have described adult and pediatric leukemia patients who exhibit MDR associated with amplification of the mdr1 gene and increased expression of P-gp (Fojo 1989, Kuwazuru et al. 1990, Mattern et al. 1989). Therefore, the question of whether MDR tumor cells remain susceptible to cell-mediated lysis bears considerable clinical significance. The present study was undertaken to examine this question.
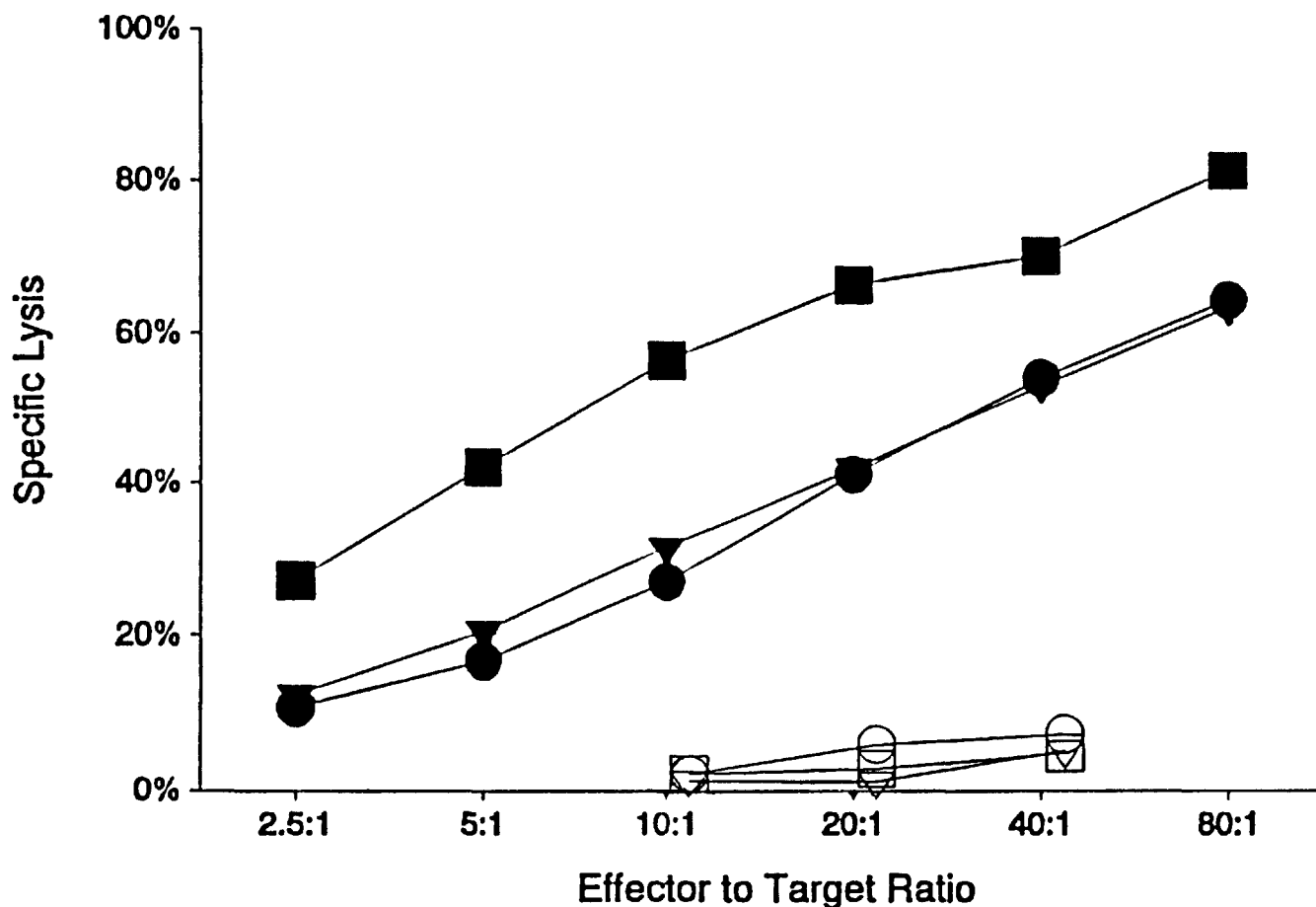
Results of the present study clearly demonstrate that an

FIGURE 1. Comparison of the susceptibility of RAJI (●) and the MDR variants R/ADR (■) and R/VP-16 (▼) to lysis by NK cells (open symbols) and LAK cells (solid symbols). Unstimulated peripheral blood lymphocytes were separated by ficoll-hypaque density centrifugation and tested directly as effectors of NK lytic activity against 10,000 or 20,000 ^{51}Cr-labeled lymphoma targets. Peripheral blood lymphocytes from the same donors were stimulated for five days with 100 IU/ml IL-2 and used as LAK effectors. After four hours incubation at 37°C, aliquots were assayed to determine specific ^{51}Cr-release. Cytotoxicity is expressed as the average percentage of specific lysis calculated as described in Materials and Methods. Each point represents the mean of determinations with three normal donors, each set up in triplicate; standard deviations were always less than 5%.

MDR phenotype does not invariably confer resistance to the cytolytic activity of LAK cells. The P-gp-negative, MDR variant R/VP-16 was found to be as susceptible to LAK lysis as the parent RAJI lymphoma line (Table 1, Fig. 1). Moreover, the P-gp-positive variant R/ADR was nearly 8-times more susceptible than the parent line.

The present results contrast with recent reports showing an inverse relationship between the level of P-gp expression and the susceptibility to LAK lysis of human leukemic cell lines (Kimmig et al. 1990). Instead, our results parallel the findings of Allavena et al. (1987) who reported that LAK cells lyse MDR human colon adenocarcinoma cell lines at least as well as the parental cell line. As in our model system, their "drug-sensitive" parental cell line (LoVo/H) expressed low levels of P-gp (Rivoltini et al. 1990). This low level expression suggests that the parent lines are themselves somewhat drug-resistant and thus makes it difficult to draw firm conclusions regarding the susceptibility of parental versus "drug-resistant" cells. Rivoltini et al. (1990) have demonstrated that the P-gp molecule does not directly alter target LAK susceptibility. However, ADR-selected P-gp-positive variants may harbor additional alterations which contribute to the drug resistance

phenotype (Baas et al. 1990, Deffie et al. 1988). Such as-yet unidentified alterations may also play a role in the increased LAK sensitivity of R/ADR.

In vitro incubation of NK cells with IL-2 increases surface expression of the cell adhesion molecules LFA-1 (CD11a/CD18), CD2, ICAM1 (CD54), LFA-3 (CD58) and the NK cell-associated antigen NKH-1 (CD56) (Robertson et al. 1990). Altered sensitivity of target cells to NK or LAK lysis, therefore, might be associated with changes in the expression of the ligands of these adhesion molecules. Scheper et al. (1991) recently reported that LFA-3 and ICAM-1 expression was reduced in two P-gp-positive MDR sublines. However, in the current study immunofluorescence analyses revealed no differences in percent positive cells or in fluorescence intensity between RAJI cells and the two MDR sublines. These results indicate that an increase (R/ADR) or decrease (R/VP-16) in P-gp expression does not invariably lead to altered expression of the adhesion molecules known to be associated with NK/LAK lysis.

Parent and MDR RAJI lines were resistant to NK lysis; this resistance may result from the strong expression on all cells of MHC class I molecules, which have been shown

to inhibit NK cell function in certain model systems (Storkus and Dawson 1991). The equivalent expression of adhesion molecules by R/ADR and RAJI suggests that the elevated LAK susceptibility of R/ADR is probably not a result of enhanced conjugate formation between targets and effector cells. Perhaps R/ADR cells more readily activate the LAK lytic pathway or are more susceptible to the cytotoxic factors released by LAK effectors.

The results of the current study support the use of immunotherapy as a potentially valuable treatment modality. Further studies are needed to explain the underlying basis for the variable LAK sensitivity of MDR cells that has been reported by various investigators. Characterization of such paired sensitive/resistant cell lines may ultimately contribute to our basic understanding of the mechanism by which NK/LAK cells recognize and kill their tumor targets.

LITERATURE CITED

Adler, A., P. A. Chervenick, T. L. Whiteside, E. Lotzova, and R. B. Herberman 1988 Interleukin 2 induction of lymphokine-activated killer (LAK) activity in the peripheral blood and bone marrow of acute leukemia patients. I. Feasibility of LAK generation in adult patients with active disease and in remission. Blood 71: 709-716.

Allavena, P., M. Grandi, M. D'Incalci, 0. Geri, F. C. Giuliani, and A. Mantovani 1987 Human tumor cell lines with pleiotropic drug resistance are efficiently killed by interleukin-2 activated killer cells and by activated monocytes. Int. J. Cancer 40: 104-107.

Baas, F., A. P. M. Jongsma, H. J. Broxterman, R. J. Arceci, D. Housman, G. L. Scheffer, A. Riethorst, M. van Groenigen, A. W. M. Nieuwint, and H. Joenje 1990 Non-P-glycoprotein mediated mechanism for multidrug resistance precedes P-glycoprotein expression during in vitro selection for doxorubicin resistance in a human lung cancer cell line. Cancer Res. 50: 5392-5398.

Beck, W. T., M. C. Cirtain, M. K. Danks, R. L. Felsted, A. R. Safa, J. S. Wolverton, D. P. Suttle, and J. M. Trent 1987 Pharmacological, molecular and cytogenetic analysis of "atypical" multi-drug-resistant human leukemic cells. Cancer Res. 47: 5455-5460.

Curt, G. A., N. J. Clendeninn, and B. A. Chabner 1984 Drug resistance in cancer. Cancer Treat. Rep. 68: 87-99.

Danks, M. K, J. C. Yalowich, and W. T. Beck 1987 Atypical multiple drug resistance in a human leukemic cell line selected for resistance to teniposide (VM-26). Cancer Res. 47: 1297-1301.

Deffie, A. M., T. Alam, C. Seneviratne, S. W. Beenken, J. K. Batra, T. C. Shea, W. D. Henner, and G. J. Goldenberg 1988 Multifactorial resistance to adriamycin: Relationship of DNA repair, glutathione transferase activity, drug efflux, and P-glycoprotein in cloned cell lines of adriamycin-sensitive and -resistant P388 leukemia. Cancer Res. 48: 3595-3602.

Fojo, A. T. 1989 Multidrug resistance to chemotherapy. Cancer Bull. 41: 26-30.

Glisson, B., R. Gupta, S. Smallwood-Kentro, and W. Ross 1986

Characterization of acquired epipodophyllotoxin resistance in a Chinese hamster ovary cell line: Loss of drug-stimulated DNA cleavage activity. Cancer Res. 46: 1934-1938.

Grimm, E. A., A. Mazumder, H. Z. Zhang, and S. A. Rosenberg 1982 Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumour cells by interleukin-2-activated autologous human peripheral blood lymphocytes. J. Exp. Med. 155: 1823-1841.

Harker, W.G., C. Tom, J. R. McGregor, L. Slade, and W. E. Samlowski 1990 Human tumor cell line resistance to chemotherapeutic agents does not predict resistance to natural killer or lymphokine-activated killer cell-mediated cytolysis. Cancer Res. 50: 5931-5936.

Higuchi, C. M., J. A. Thompson, T. Cox, C. G. Lindgren, C. D. Buckner, and A. Fefer 1989 Lymphokine-activated killer function following autologous bone marrow transplantation for refractory hematological malignancies. Cancer Res. 49: 5509-5513.

Kartner, N., D. Evernden-Porelle, B. Bradley, and V. Ling 1985 Detection of P-glycoprotein in multidrug-resistant cell lines by monoclonal antibodies. Nature 316: 820-823.

Kimmig, A., V. Gekeler, M. Neumann, G. Frese, R. Handgretinger, G. Kardos, H. Diddens, and D. Niethammer 1990 Susceptibility of multidrug-resistant human leukemia cell lines to human interleukin 2-activated killer cells. Cancer Res. 50: 6793-6799.

Kuwazuru, Y., A. Yoshimura, S. Hanada, A. Utsunomiya, T. Makino, K. Ishibashi, M. Kodama, M. Iwahashi, T. Arima, and S.-I. Akiyama 1990 Expression of the multidrug transporter, P-glycoprotein, in acute leukemia cells and correlation to clinical drug resistance. Cancer 66: 868-873.

Lotzova, E., C. A. Savary, R. E. Pollock, and P. Fuchshuber 1990 Immunologic and clinical aspects of natural killer cells in human leukemia. Nat. Immun. Cell. Growth Regul. 9: 173-181.

Mattern, J., T. Efferth, M. Bak, A. D. Ho, and M. Volm 1989 Detection of P-glycoprotein in human leukemias using monoclonal antibodies. Blut 58: 215-217.

Ohtsu, A., Y. Sasaki, T. Tamura, Y. Fujiwara, Y. Ohe, K. Minato, K. Nakagawa, M. Bungo, and N. Saijo 1989 Inhibition of colony formation of drug-resistant human tumor cell lines by combinations of interleukin-2-activated killer cells and antitumor drugs. Jpn. J. Cancer Res. 80: 265-270.

Rivoltini, L., M. P. Colombo, R. Siupino, D. Ballinari, T. Tsuruo, and G. Parmiani 1990 Modulation of multidrug resistance by verapamil or mdr1 anti-sense oligodeoxynucleotide does not change the high susceptibility to lymphokine-activated killers in mdr-resistant human carcinoma (LoVo) line. Int. J. Cancer 46: 727-732.

Robertson, M. J., M. A. Caligiuri, T. J. Manley, H. Levine, and J. Ritz 1990 Human natural killer cell adhesion molecules: Differential expression after activation and participation in cytolysis. J. Immunol. 145: 3194-3201.

Scheper, R. J., W. S. Dalton, T. M. Grogan, A. Schlosser, W. T. Bellamy, C. W. Taylor, P. Scuderi, and C. Spier 1991 Altered expression of P-glycoprotein and cellular adhesion molecules on human multidrug-resistant tumor cells does not affect their susceptibility to NK- and LAK-mediated cytotoxicity. Int. J. Cancer 48: 562-567.

Scudiero, D. A., R. H. Shoemaker, K. D. Paul, A. Monks, S. Tierney, T. H. Nofziger, M. J. Currens, D. Seniff, and M. R. Boyd 1988 Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res. 48: 4827-4833.

Singh, S. V., S. Nair, H. Ahmad, Y. C. Awasthi, and A. Krishan 1989 Glutathione-S-transferases and glutathione peroxidases in doxorubicin-resistant murine leukemic P388 cells. Biochem. Pharmacol. 38: 3505-3510.

Stong, R. C., F. Uckun, R. J. Youle, J. H. Kersey, and D. A. Vallera 1985 Use of multiple T cell-directed intact ricin immunotoxins for autologous bone marrow transplantation. Blood 66: 627-635.

Storkus, W. J and J. R. Dawson 1991 Target structures involved in natural killing (NK): Characteristics, distribution, and candidate molecules. Crit. Rev. Immunol. 10: 393-416.