Resistance of Human Ovarian Cancer Cells to Tumor Necrosis Factor and Lymphokine-activated Killer Cells: Correlation with Expression of HER2/neu Oncogenes

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ABSTRACT

Since overexpression of HER2/neu oncogenes in breast cancer cells is associated with resistance to the cytotoxic effect of tumor necrosis factor (TNF), we investigated whether this correlation also existed for ovarian cancer targets. Nine continuously cultured human ovarian cancer lines were studied and compared to 3 breast cancer lines. Three of the ovarian and 1 breast cancer line demonstrated amplified HER2/neu genes by Southern analysis, increased HER2/neu RNA by Northern analysis, and marked immunoperoxidase staining for HER2/neu protein. The other 8 lines contained unamplified genes and undetectable RNA and protein. All 4 overexpressed lines were relatively resistant to the cytotoxic effects of TNF. Interestingly, they were also resistant to lymphokine-activated killer cells. In contrast, 7 of 8 nonexpressed lines showed sensitivity to TNF and all 8 were sensitive to lymphokine-activated killer cells. There was no difference in sensitivity to lysis by hydrogen peroxide or peptide defensins between over- and nonexpressed lines. These data indicate that expression of HER2/neu oncogenes may impart a proliferative advantage in tumor cells due to induction of resistance to several different cytotoxic mechanisms.

INTRODUCTION

Recent work suggests that the HER2/neu oncogene (also known as c-erbB-2) plays an important role in carcinogenesis. The gene is amplified in approximately 30% of primary breast cancers (1) and overexpression is associated with aggressive behavior (1). Furthermore, amplified expression of HER2/neu in transfected 3T3 cells induces malignant transformation (2, 3).

In addition, to an increased inherent proliferative potential, resistance to host defense mechanisms may also play a role during in vivo growth of HER2/neu-overexpressed tumors. Amplified expression in transfected cells correlates with resistance to TNF3 (4), an important effector molecule of tumoricidal macrophages. Furthermore, a correlation exists between HER2/neu expression and TNF resistance in continuously passaged breast tumor lines (4, 5).

Another human tumor system in which HER2/neu may be important is ovarian cancer. A significant number of fresh specimens (20–30%) contain HER2/neu RNA and protein (6) and overexpression also correlates with a poor prognosis (6).

The sensitivity of ovarian cancer cells to TNF-induced (7) and macrophage-mediated (8) cytotoxicity is heterogeneous. We, thus, investigated whether this variable sensitivity to lytic mechanisms was also related to variable oncogene expression. The results of this study with continuously cultured ovarian cancer lines confirm the correlation between HER2/neu and TNF resistance and indicate that this phenomenon is not peculiar to breast cancer targets. Of further interest, HER2/neu-overexpressed ovarian and breast cancer cells were also relatively resistant to lysis by IL-2-activated lymphocytes. These data suggest that the expression of HER2/neu oncogenes in human tumor cells may impart a proliferative advantage due to induction of resistance to several different host cytotoxic mechanisms.

MATERIALS AND METHODS

Cell Lines. The HBL-100 and the MCF-7 breast cancer lines were generous gifts of Dr. D. Slamon, UCLA. SKOV3, PA-1, Caov3, OVCAR-3, SRO-82, and SKBR3 tumor lines were obtained from the American Type Culture Collection (Rockville, MD). Ovarian cancer lines 204, 222, 194, and 436 were established by us in culture after isolation from peritoneal fluid or solid tumors from patients with ovarian cancer. They have now demonstrated stable growth for >1 year. The initiation of these lines has been previously described (7). All ovarian lines were maintained as monolayer cultures in RPMI 1640 media supplemented with 10% FCS (GIBCO, Grand Island, NY), 1% sodium pyruvate, 1% nonessential amino acids, 1% t-glutamine, and 1% penicillin-streptomycin. The SKBR3 and HBL-100 breast cancer lines were continuously cultured in Iscove’s modified media (GIBCO) supplemented with 10% FCS. The MCF-7 line was cultured in Dulbecco’s modified essential media supplemented with 10% FCS and 5 μg/ml insulin. Tumor cells were harvested by overlaying the monolayer with a solution of 0.25% trypsin and 0.01% EDTA.

Reagents. Human rTNF was a generous gift from Genentech (South San Francisco, CA). The specific activity was 4.78 × 107 units/mg protein. The three human defensins (HNP 1, 2, and 3) were purified to homogeneity from normal human granulocytes as previously described (9) and used in a ratio of 2:2:1. Human recombinant IL-2 was purchased from Cellular Products (Buffalo, NY).

MTT Assays. The MTT assay was performed as previously described (10). Briefly, targets were suspended in RPMI and 5% FCS and 100 μL of the cell suspension was dispensed into 96-well microtiter plates. Adherent cells were allowed to attach by overnight incubation at 37°C. Serial dilutions of rTNF were then added to the wells in volumes of 100 μL. Triplicate wells were run for each group and 4–6 wells were run containing target cells without TNF as a control for cell viability. Six wells without targets but containing tissue culture media were used to control for nonspecific dye reduction which was always <0.003 OD units. After varying intervals of culture at 37°C, the plates were removed from the incubator and an aliquot of 20 μL of MTT (Sigma Chemical Co., St. Louis, MD) stock solution (5 μg/ml) was added to each well followed by incubation at 37°C for 4 h. The unreacted dye and medium was then removed and the MTT formazan was solubilized by addition.
of 100 μl of acidified isopropanol alcohol. The absorbance of each well was measured with a microplate enzyme-linked immunosorbent assay reader equipped with a 570-nm filter. The reader was calibrated to 0 absorbance using wells containing only medium and MTT. To assay the effects of cytostasis and cytolyis, *5 × 10^3* targets were initially seeded/well. To solely measure cytolyis, *2 × 10^4* targets with 0.5 μg/ml mitomycin-C were seeded/ well. The absorbance of control wells containing *2 × 10^4* targets with mitomycin-C was consistently between 0.3 and 0.5. After 72 h in culture, the absorbance of control wells initially containing *5 × 10^3* targets was usually between 0.2 and 0.4. The SD of replicate samples was always <5% of the mean. The results of the MTT assay are presented as relative percentage of survival determined as follows:

\[
\text{OD-exp group} \times 100
\]

Each experiment was repeated at least 3 times. In addition, an LD₉₀ for TNF was determined by extrapolation from the results of the MTT assay.

Chromium Release Assay. Targets (*10^7*) were incubated with 50 μCi ^51Cr for 1 h at 37°C. Labeled targets (*10^3* in 0.1 ml) were added to 0.1 ml of increasing concentrations of TNF, defensins (1–100 μg/ml), or increasing numbers of LAK cells in microtiter plates. All assays were performed in RPMI and 5% FCS except for defensin experiments in which no FCS was used. After incubation at 37°C for varying durations, the plates were centrifuged and 0.1 ml of supernatant was counted in a gamma counter. Percentage of lysis was calculated as:

\[
\frac{(\text{cpm-exp} - \text{cpm-control})}{(\text{cpm-maximal} - \text{cpm-control})} \times 100
\]

Control release was determined from targets incubated in media alone and maximal release was achieved by adding 1 ml HCl (>90% incorporated counts). Control isotope release at 20 h ranged from 23–34% for the various targets. The assays were performed in quadruplicate and the SD of replicates was always <5% of the mean. The LD₉₀ was determined for H₂O₂ and defensins by interpolation from their lysis versus concentration curves as previously described (11) and presented as the geometric mean of at least 3 experiments. The LAK cells were generated by incubating peripheral blood lymphocytes (10^6/ml) in AIM-V media with 50 or 100 units of rIL-2/ml for 4 or 6 days after which the cells were washed 3 times.

Probes. The HER2/neu probe is an 897-base pair Sphi-EcoRI fragment containing the 3' end of the coding sequence and is subcloned in pBR322 (12). The β-TCR probe is a 0.4-kilobase BglII-BglII insert in the plasmid Bluescribe, subcloned from the JUR-β complementarY DNA clone (13). The chromosome 17 pYNZ22 probe is a 1.7-kilobase BamHI fragment from the cosmID YNZ22 subcloned into pBR322 (14). The mouse α-actin probe which cross-hybridizes with human α-actin is a 0.9-kilobase PstI-PstI insert in pUC8 (15).

Southern Blot Analysis. Genomic DNA was analyzed by Southern blot analysis as previously described (16). Parallel lanes of tumor cell and granulocyte (germ line) DNA were hybridized with the HER2/neu probe and single copy control probes (β-TCR or pYNZ22) to determine that similar amounts of DNA were loaded in each lane. Densitometric scanning of autoradiographic signals was performed by a Beckman DU-8 scanning spectrophotometer. Densitometric analysis was carried out [as described previously (16)] to more precisely determine the amount of amplification by comparing the oncogene hybridization signal in diluted amounts of tumor DNA to undiluted granulocyte DNA.

Northern Blot Analysis. RNA was analyzed as previously described (16). Twenty-five μg of total cellular RNA from the tumor lines were electrophoresed on formaldehyde agarose gels. The RNA samples were transferred to nylon filters and hybridized with the HER2/neu or an α-actin control probe to ensure that intact RNA was loaded in each lane.

Immunoperoxidase. Immunoperoxidase staining was performed as previously described (17). Cytocentrifuge preparations were air dried and fixed in fresh acetone for 5 min at 25°C. Slides were rehydrated in phosphate-buffered saline for 10 min and incubated with c-erbB-2 monoclonal antibody (Triton Biosciences, Alameda, CA) at 25°C for 30 min. The slides were subsequently incubated with biotinylated anti-mouse IgG (Vector Labs, Inc., Burlingame, CA) at 1:200 for 30 min at 25°C. Following incubation with secondary antibody, the slides were reacted with an avidin-horseradish peroxidase complex (Vector Labs) for 15 min. The substrate, amino-ethyl carbazole (red precipitate), was then applied. After the slides were washed in water, they were counterstained for 3 min in Mayer's hematoxylin and mounted in an aqueous mounting medium. Control slides included omission of the primary antibody. No background staining was observed.

Statistics. The t test was used to determine P values.

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**RESULTS**

Southern, Northern, and Immunoperoxidase Analysis. Nine continuously cultured ovarian lines were studied. For comparison, three breast lines whose HER2/neu status was already known (5) were also studied. All the ovarian lines were derived from epithelial adenocarcinomas except for PA-1 which is a germ cell tumor (Table 1). All grew as adherent lines with doubling times that ranged from 30–80 h. Southern blot analysis demonstrated that three of the ovarian cancer lines (SKOV3, 194, and 436) contained an identical rearrangement of the HER2/neu oncogene. In addition to the germ line HER2/neu fragment, EcoRI-, BamHI-, and BclI-digested DNA showed a rearranged fragment. The details of this rearrangement will be presented in a separate publication. Since HindIII-digested DNA did not reveal the rearrangement, DNA restricted with this enzyme was used to determine the degree of gene amplification. All three lines with the rearrangement (SKOV3, 194, and 436), in addition to the SKBR3 line, demonstrated amplification of the HER2/neu gene (Fig. 1). Densitometric and dilutional analysis determined that these lines contained a 4- to 8-fold increase in copy number. In contrast, the remaining six ovarian and two breast lines contained single copies of this gene. This was confirmed by running granulocyte germ line controls (not shown) with each of the eight lines and demonstrating hybridization signals with the HER2/neu probe which were similar to the tumor lines. Fig. 1 shows the Southern analyses of six of these eight lines (not shown are analyses of Caov3 and HBL-100 which also demonstrated single copies of the oncogene). Equivalency of the DNA loaded in each lane was confirmed by similar hybridization signals when the blot was rehybridized with the control β-TCR probe (Fig. 1). The increase in HER2/neu copy number for SKBR3, SKOV3, 194, and 436 cells was the result of gene amplification and not simply chromosome reduplication since another chromosome 17 probe, pYNZ22 (14), demonstrated results similar to the TCR probe.

Northern analysis demonstrated marked expression of HER2/neu in the four lines that contained amplified genes. Moreover, the three lines with rearrangements (SKOV3, 194,
Fig. 1. Granulocyte (germ line control) and tumor cell DNA blot hybridization with the HER2/neu and \( \beta \)-T-cell receptor (\( \beta \)-T) probes. All lanes were loaded with 10 \( \mu \)g of HindIII-digested DNA. The tumor line (1–10) and granulocyte (C) DNAs shown are from five separate filters. The numbers correspond to the following tumor lines: 1, SKOV3; 2, SKBR3; 3, 436; 4, 194; 5, 204; 6, OVCAR-3; 7, SRO-82; 8, 222; 9, MCF-7; 10, PA-1. kb, kilobases.

436) also demonstrated expression of an aberrant 12-kilobase transcript in addition to the normal 4.5-kilobase transcript (Fig. 2). This aberrant transcript was present at markedly higher levels than the normal RNA species in all three of these lines. Not shown in Fig. 2 is the enhanced expression of the 4.5-kilobase transcript in SKBR3 cells which has also been previously described (4, 5). By contrast, none of the other eight lines demonstrated significant expression even though the intactness of the RNA and equivalency of loading was demonstrated by showing similar hybridization signals with the \( \alpha \)-actin control probe. Fig. 2 shows the Northern analyses of seven of the eight nonexpressed lines (not shown is analysis of SRO-82 which similarly showed lack of expression).

Immunoperoxidase assay for the protein gene product was also consistent with the above studies. All four lines with increased copy number of the HER2/neu gene, and increased levels of transcripts stained brightly in a predominant membrane pattern. Intense staining of these lines was present when monoclonal antibody was used at dilutions of 1:30–1:120. Staining began to gradually decrease at higher dilutions but remained detectable at dilutions >1:1000. Although there was some heterogeneity in the intensity of immunoperoxidase staining among the positive tumor cell populations, >99% of the cells were clearly stained. No staining of the other eight lines was detected even when monoclonal antibody was used at a 1:10 dilution. Figs. 3 and 4 demonstrate examples of a HER2/neu-positive and -negative line stained by the immunoperoxidase technique.

Sensitivity to TNF. In a 72-h MTT dye assay, the six nonexpressing ovarian lines (open symbols, Fig. 5) were all comparable in their sensitivity to TNF. In contrast, all three of the overexpressing ovarian lines (closed symbols, Fig. 5) were resistant to TNF.
HER2/neu GENES AND RESISTANCE TO CYTOTOXICITY

 symbols, Fig. 5), using concentrations up to 12 nm (10,000 units/ml). Even after 96 h of incubation with TNF, inhibition never exceeded 10%. All three overexpressed targets demonstrated growth enhancement at most concentrations of TNF in a similar fashion to what other investigators have shown (18). Three of the six nonexpressed lines demonstrated modest growth enhancement at the lowest concentrations of TNF (1.2 and 12 pm). For all TNF concentrations between 60 and 1.2 x 10^6 pm, each of the three overexpressing lines demonstrated relative survival which was outside 2 SD of the mean values calculated for the six nonexpressing lines.

Table 2 shows that several of the nonexpressing lines were more sensitive to TNF after a 72-h incubation when compared to a 24-h assay. Thus, all six ovarian lines that did not express HER2/neu oncogenes were significantly sensitive to cytotoxic action of TNF in a concentration- and time-dependent fashion. Table 2 also shows that resistance to TNF in HER2/neu-positive ovarian lines is not part of a nonspecific resistance to all lytic mechanisms. The concentration of H2O2 inducing 50% lysis was very similar among nonexpressing (3 x 10^-3-2 x 10^-3 M) and overexpressing (5 x 10^-3-3 x 10^-3 M) lines. Also, the concentration of peptide defensins (9) required to lyse 50% of overexpressing targets (25-40 μg/ml) was comparable to that of the nonexpressing lines (30-60 μg/ml).

For comparison purposes, the sensitivity of the three breast lines to TNF was also studied. Fig. 6 demonstrates that the nonexpressing HBL-100 breast line was resistant, while the nonexpressing MCF-7 line was sensitive, to TNF. The overexpressing SKBR3 line was resistant to all concentrations of TNF. The survival of MCF-7 cells was significantly decreased (P < 0.05) at all concentrations of TNF (except at 1.2 pm) when compared to the other two lines. These data are consistent with previously published results (5). Resistance of SKBR3 and HBL-100 cells was also seen when assays were prolonged to 96 h. Both resistant lines demonstrated growth enhancement at all concentrations of TNF similar to what was seen with the resistant ovarian lines. As with the ovarian lines, there were no significant differences in LD50 values for H2O2 or defensins between over- and nonexpressing breast lines (Table 2).

When we performed the MTT assay in the presence of mitomycin-C to abrogate any cytostatic effect of TNF, little

Table 2 Concentrations of TNF, H2O2, and defensins inducing 50% lysis

Tumor targets were incubated with increasing concentrations of TNF, reagent grade H2O2, or defensins and % survival (for TNF) or % lysis (for H2O2 or defensins) was determined as described in "Materials and Methods." LD50 values were calculated by interpolation from curves. Results are geometric means of at least 3 experiments. SEM was always <5% of the means. TNF assays were incubated for 24 or 72 h; H2O2 and defensins assays were incubated for 20 h.

<table>
<thead>
<tr>
<th>Target</th>
<th>TNF-24 h (pm)</th>
<th>TNF-72 h (pm)</th>
<th>H2O2 (mM)</th>
<th>Defensins (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>222</td>
<td>630</td>
<td>300</td>
<td>1.2</td>
<td>40</td>
</tr>
<tr>
<td>204</td>
<td>1400</td>
<td>1000</td>
<td>0.33</td>
<td>55</td>
</tr>
<tr>
<td>SRO-82</td>
<td>500</td>
<td>12</td>
<td>0.6</td>
<td>NT</td>
</tr>
<tr>
<td>PA-1</td>
<td>1000</td>
<td>600</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>OVCAR</td>
<td>1800</td>
<td>1200</td>
<td>0.9</td>
<td>50</td>
</tr>
<tr>
<td>CAOV3</td>
<td>1400</td>
<td>1000</td>
<td>NT</td>
<td>60</td>
</tr>
<tr>
<td>194</td>
<td>0.8</td>
<td>25</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>436</td>
<td>0.49</td>
<td>20</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>SKOV3</td>
<td>3</td>
<td>25</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKBR3</td>
<td>1</td>
<td>25</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>MCF-7</td>
<td>0.8</td>
<td>30</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>HBL-100</td>
<td>1.4</td>
<td>20</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

* NT, not tested.

There is no significant difference (P > 0.05) in LD50 values for H2O2 or defensins lysis between the four overexpressed lines (SKOV3, 194, 436, SKBR3) and the mean values of the other nonexpressed lines.
change was seen in the pattern of responses. The four lines that overexpressed HER2/neu oncogenes as well as the nonexpressing HBL-100 breast line remained consistently resistant to TNF (cytotoxicity <10% at all concentrations of TNF up to 12 nM and between 24–72 h of incubation). The other nonexpressing lines were still significantly sensitive, although their LD50 values were somewhat higher (mean, 11 nM) as compared to when the assay was run without mitomycin-C (mean, 1 nM). Since the survival of these lines was significantly inhibited even when cell proliferation was prevented with mitomycin-C, these data suggest they are sensitive to the cytolytic action of TNF, although a cytostatic effect may also occur when the assay is performed without mitomycin-C. Chromium release assays performed after 20 h of incubation with TNF confirmed this notion. Specific lysis of all nonexpressing lines except HBL-100 reached 25–40% at TNF concentrations >0.5 nM. It is possible that lysis could have been greater with longer incubations but excessive spontaneous isotope release precluded testing of targets beyond 20 h. In contrast to these results, the four overexpressing lines as well as HBL-100 remained resistant (lysis <10% at all TNF concentrations up to 12 nM).

Sensitivity to LAK Cells. Eleven of the 12 lines (204 was omitted) were tested for sensitivity to lysis mediated by IL-2-activated lymphocytes (4-day culture with 50 units/ml rIL-2) in a 3-h chromium release assay (Fig. 7). None of these targets were sensitive to nonactivated natural killer lymphocytes. A surprising correlation between LAK lysis and HER2/neu expression was detected. Of the four overexpressed lines, only lysis of SKBR3 breast targets reached levels >30% at the highest E:T ratio tested and this was significantly less than that seen with all seven nonexpressing lines. Lysis of the other three overexpressed lines was never >20% at 50:1 E:T ratios. Interestingly, the HBL-100 line, which did not express HER2/neu genes but was completely resistant to TNF, was the most sensitive to LAK cells. Two other experiments in which the conditions of LAK cell generation were altered (100 units/ml rIL-2 and 6 days of culture) produced identical results.

DISCUSSION

Four of 12 ovarian and breast cancer cell lines containing amplified HER2/neu genes, increased levels of HER2/neu RNA, and increased expression of HER2/neu protein were resistant to the cytotoxic action of TNF and IL-2-activated lymphocytes. Since overexpressed lines were equally sensitive to lysis by H2O2 and defensins, their resistance to TNF and LAK cells is not part of a generalized, nonspecific ability to counter all forms of cell injury. In preliminary studies, Western blots demonstrated a normal-sized HER2/neu protein species in the overexpressed lines that contain an aberrant RNA species. In addition, overexpressed SKBR3 cells as well as transfected 3T3 cells (4) contain unarranged genes, normal RNA species, and normal-sized protein and are also resistant to TNF. These data suggest that overexpression of a normal HER2/neu protein is sufficient for resistance to these cytotoxic mechanisms.

Our results confirm and extend the work of Hudziak et al. (4) and Shepard and Lewis (5). These authors reported that resistance to the cytotoxic action of TNF correlated with overexpression of HER2/neu oncogenes in several continuously cultured breast cancer cell lines. We now show that this correlation can be extended to a second human tumor system in which HER2/neu expression is relatively common, ovarian cancer. In addition, the unexpected correlation of oncogene expression with resistance to LAK-induced cytotoxicity has not been previously noted.

The effects of TNF on target cells can be cytolysis, cytostasis, or, under some conditions, growth stimulation. The results of chromosome release and MTT assays which include mitomycin-C indicate that the nonexpressing lines were clearly lysed and such lysis occurred by 24 h. Since, at 48 and 72 h, MTT assays without mitomycin-C generally resulted in lower LD50 values when compared to those performed with mitomycin-C, cytostasis of sensitive target cells that initially survived lytic attack during the first 24 h may have also occurred. In contrast, HER2/neu-overexpressed lines were resistant to cytolysis as well as cytostasis. While low concentrations of TNF significantly stimulated the proliferation of three of the six sensitive ovarian lines, the TNF-resistant lines were growth stimulated at all concentrations tested. These data are consistent with a previous study (18) which also showed that targets rendered resistant to the cytotoxic action of TNF retain their ability to
respond with enhanced growth at some concentrations. Thus, the responses of growth stimulation and growth inhibition in a single target cell can be dissociated, suggesting that they are mediated by distinct intracellular pathways.

The study by Hudziak et al. (4) suggested that resistance associated with HER2/neu expression was due to an altered TNF receptor with decreased avidity. Other investigators, studying different models of TNF resistance in different targets, have implicated the presence of gap junctions between cells (19), an increased antioxidant activity of the glutathione cycle (20), the activity of superoxide dismutase (21), and the expression of TNF genes (22) and production of TNF protein (23).

Preliminary experiments on these HER2/neu-overexpressed lines indicate that resistance can be significantly reversed by coinubcation with nontoxic concentrations of actinomycin-D or cycloheximide. Whether these drugs sensitize targets to TNF by preventing endogenous synthesis of TNF, superoxide dismutase, or other “protective proteins” remains to be seen. It will also be interesting to see whether similar treatment with these protein synthesis inhibitors can sensitize overnight exposed targets to lysis by LAK cells.

In addition to the correlation between oncogene expression and TNF resistance, we were surprised to detect a further association with resistance to LAK cell cytotoxicity. Recent studies indicate that murine cytotoxic T-lymphocytes maintained in IL-2 (24, 25) and human IL-2-activated cytotoxic lymphocytes (26-28) can be stimulated to release TNF or a TNF-like protein with cytotoxic capability. This suggests two possible explanations for the correlation with resistance to LAK cytotoxicity. First, LAK killing of ovarian and breast cancer targets may actually be mediated by TNF released from IL-2-activated effectors. Thus, any HER2/neu-overexpressed TNF-resistant line would also be resistant to LAK cells. Second, LAK cell lysis of these targets may be mediated by other cytotoxins released by IL-2-activated lymphocytes but susceptibility to lysis may be greatly enhanced by coexposure to TNF which is also released by effectors. TNF has been shown to sensitize targets to LAK cytolsis under some conditions (29).

Thus, resistance to TNF in HER2/neu-overexpressed lines might also correlate with resistance to this sensitizing effect of TNF on LAK cytotoxicity. However, the lysis induced by TNF or TNF-like protein secreted by cytotoxic lymphocytes demonstrates relatively slow kinetics (25, 28) and LAK cell lysis of nonexpressing tumors in our study was virtually complete by 3–4 h. In experiments not shown, even when we exposed nonexpressing targets to 10,000 units/ml of rTNF, there was no lysis after only 3 h of incubation. It is, therefore, unlikely that our LAK cells kill targets by releasing TNF. In addition, if simple resistance to TNF prevents LAK cell lysis because TNF is the effector molecule or is a critical sensitizer for lysis by other mediators, then the TNF-resistant HBL-100 breast line, which does not express HER2/neu genes, should also have been resistant to LAK cells. Its clear sensitivity argues against these hypotheses. Instead, a third possibility is more likely, namely, that there is some important event common to the pathways of TNF- and LAK cell-mediated lysis which is regulated by target cell HER2/neu gene expression. Thus, future study of HER2/neu-overexpressed targets offers great possibilities for gaining insight into the mechanisms of both lytic effector systems.

It remains to be determined whether these results with continuously cultured cells lines can be generalized to human cancer growing in patients. Approximately 30% of freshly obtained ovarian cancer cells contain overexpressed HER2/neu genes (6) and primary explants are variably resistant to TNF (7), cytotoxic macrophages (8), and LAK cells (30). Resistance to both macrophage-released TNF and IL-2-activated lymphocytes would provide a significant advantage for in vivo growth of such tumors in patients. Obviously, attempted correlations between gene expression and sensitivity to cytotoxic mechanisms in fresh explants would be of great interest.

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