Interferon-induced Increase in Sensitivity of Ovarian Cancer Targets to Lysis by Lymphokine-activated Killer Cells: Selective Effects on HER2/neu-overexpressing Cells

Catherine Fady, Agnes M. Gardner, Joseph F. Géra, and Alan Lichtenstein

Department of Medicine, VA Wadsworth-UCLA Medical Center, the Wadsworth VA Hospital and the Jonsson Comprehensive Cancer Center, Los Angeles, California 90073

Abstract

Overexpression of the HER2/neu onco gene in ovarian tumor cells is associated with relative resistance to lymphokine-activated killer (LAK) cell cytotoxicity. Treatment with γ-interferon (IFN-γ) (200–2000 units/ml) for 3 days markedly enhanced the sensitivity of HER2/neu-overexpressing ovarian tumor cells to LAK cells but had no effect on the sensitivity of nonexpressing ovarian targets. Increased sensitivity to lysis was associated with an increase in effector-target conjugate formation, the induction of target cell intercellular adhesion molecule 1 (ICAM-1) expression, and the down-regulation of HER2/neu expression. Anti-ICAM-1 antibody blocked the enhanced lysis, indicating that ICAM-1 is important in the increased sensitivity to LAK cells. However, induction of ICAM-1 expression did not correlate well with enhanced sensitivity to lysis; it was maximal after 24 h of exposure to IFN-γ and still present 24 h after removing IFN-γ. In contrast, enhanced lysis required 3 days of exposure to IFN-γ and was reversed within 24 h after removal of IFN-γ. These data indicate that, although ICAM-1 is necessary, it is not sufficient for the IFN-γ-induced enhancement of sensitivity to LAK lysis.

Introduction

The HER2/neu onco gene is amplified and overexpressed in approximately 20–30% of human ovarian cancers (1), and its overexpression correlates with a poor prognosis. HER2/neu expression in continuously cultured ovarian cell lines, as well as in transfected targets, induces resistance to tumor necrosis factor (2), cytotoxic macrophages (2), and LAK cells (3). It is not known whether such resistance contributes to the poor prognosis associated with HER2/neu, but it may partially explain the poor results of immunotherapy in patients with ovarian cancer (only 20% partial response rate in patients treated with an i.p. injection of LAK cells and IL-2; Ref. 4).

Marth et al. (5) have shown that IFN-γ could down-regulate HER2/neu expression. In addition, other investigators have documented the occasional ability of IFN to sensitize tumor cells for lymphocyte cytotoxicity (6). These observations prompted the current study, in which we investigated whether IFN-γ could reverse the resistance of HER2/neu-overexpressing ovarian tumor cells to LAK cell lysis. The results confirm successful sensitization by IFN-γ and indicate that induction of target cell ICAM-1 determinants is necessary but not sufficient for enhanced sensitivity to LAK cells.

Materials and Methods

Tumor Cell Lines. SKOV-3, PA-1, SRO-82, CaOV-3, and OVCAR-3 ovarian cancer lines were obtained from the American Type Culture Collection (Rockville, MD). Ovarian cancer lines 204, 222, and 436 were established by us in culture after isolation from peritoneal fluid or solid tumors from patients with ovarian cancer (7). They have now demonstrated stable growth for over 1 year. All lines were maintained as monolayer cultures in complete media (RPMI 1640 media supplemented with 10% FCS (Gibco, Grand Island, NY)), 1% sodium pyruvate, 1 nonessential amino acids, 1% L-glutamine, and 1% penicillin-streptomycin). Tumor cells were harvested by overlaying the monolayer with a solution of 0.25% trypsin and 0.02% EDTA.

Cytokine Treatments. Tumor cells (1 x 10^6/ml) were treated with human recombinant IFN-γ (specific activity, 3.4 x 10^5 units/mg; Biochemical Products, Bedford, MA) in complete media for varying time intervals. After the treatment, tumor cells were trypsinized, washed, and adjusted to the appropriate concentration in RPMI + 10% FCS. In our hands, trypsinization of targets does not affect their sensitivity to LAK-induced lysis.

Human peripheral blood lymphocytes were first isolated by density gradient separation. LAK cells were then generated by culturing peripheral blood lymphocytes (10^6/ml) in AIM-V media (Gibco) containing 50 U/ml recombinant human interleukin 2 (specific activity, 1.07 x 10^5 IU/mg; Cellular Products, Buffalo, NY) for 4 to 7 days. In some experiments, LGLs were separated from bulk LAK cultures by the method of Timonen et al. (8).

Cytotoxicity Assay. Targets were labelled with 50 μCi 35Cr (DuPont-New England Nuclear, Wilmington, DE) for 1 h at 37°C and washed three times. Chromated targets (10^6 in 100 μl) were added to varying numbers of LAK cells (in 100 μl) in V-bottomed microtiter plates (Costar, Cambridge, MA). E:T ratios varied from 50:1 to 1:1, and the cytotoxicity assay was performed in RPMI with 10% FCS. After a 3-h incubation at 37°C, 100 μl of cell-free supernatant were harvested and counted in a gamma counter (Tm Analytic, Elk Grove, IL). Specific lysis was calculated as:

\[
\text{cpm(exp) - cpm(spontaneous) + cpm(max) - cpm(spontaneous)}
\]

The spontaneous release was always <25% of total incorporated counts. The maximal release, induced by adding 1% Triton X, was always >85% of incorporated counts. All groups were run in quadruplicate; the SD of each group was always <5% of the mean. In blocking studies, anti-ICAM-1 antibody (from clone 84H10; Amac, Inc., Westbrook, ME) was first dialyzed overnight on a membrane Spectra/Por 3 (Spectrum, Houston, TX) and then added directly into the wells of the cytotoxicity assay. An identically prepared and dialyzed antibody of similar isotype (IgGl) (anti-mouse keyhole limpet hemocyanin antibody; Becton Dickinson, Mountain View, CA) was used as a control.

Immunofluorescence Analysis. Cell pellets (1–5 x 10^6 cells) were treated for 30 min at 4°C with 1 μg of monoclonal antibodies. After two washes in phosphate-buffered saline, 4 μl fluorescein isothiocyanate-conjugated F(ab')2; fragment of goat anti-mouse immunoglobulins (Becton Dickinson, San Jose, CA) were added and incubated for 30 min at 4°C. After two washes, the cells were analyzed by flow cytometry using FACSscan (Becton Dickinson, Mountain View, CA). The monoclonal antibodies used were as follows: W6/32 (anti-class I HLA; Dako Patts A/S, Denmark); c-erbB-2 (anti-HER2/new; Triton Biosciences, Inc., Alameda, CA); and CD54 (anti-ICAM-1; Amac, Inc.,...
Westbrook, ME). Corresponding controls consisted of identical staining except for the absence of the primary antibody. The intensity of fluorescence was recorded on a logarithmic scale.

Conjugate Assay. LAKs (2 x 10^6/ml) and targets (2 x 10^6/ml) in RPMI and 10% FCS were first individually incubated at 30°C for 30 min. One hundred μl of LAKs were then mixed with 200 μl of targets, incubated for 5 min at 30°C, and centrifuged for 5 min at 200 rpm. The cells were then gently resuspended, at least 100 LAKs in two different areas were counted under the microscope, and the average percentage conjugation of the two areas was recorded. Effectors were easily distinguished from targets by their small size. The percentage conjugation was calculated as

Number of LAKs bound to targets

+ Total number of LAKs counted x 100

Statistical Analysis. Student’s t test was used to determine statistical significance.

RESULTS

Resistance of HER2/neu-overexpressing Target Cells to LAK-induced Cytolysis Correlates with Resistance to Effector Cell Conjugation and Absence of ICAM-1 Expression. As we have previously reported (3), the HER2/neu-overexpressing ovarian cancer cell lines SKOV-3 and 436 are relatively resistant to lysis induced by interleukin 2-activated peripheral blood lymphocytes (LAKs) when compared to nonexpressing ovarian cancer cell lines (Table 1). The HER2/neu-overexpressing SKOV-3 and 436 targets were also resistant to conjugation by LGLs fractionated from LAK cultures. This was demonstrated in a binding assay (Table 1) where conjugation was observed microscopically at the single cell level. Resistance to conjugation was also detected in other experiments (not shown) where different E:T ratios were utilized (1:1 and 1:4). The resistance of HER2/neu-overexpressing cells to both cytotoxicity and effector cell binding correlated with the lack of expression of the adhesion molecule ICAM-1 (Table 1).

The effector cells mediating cytolysis of both HER2/neu-overexpressing (HER2/neu*) and nonexpressing (HER2/neu~) targets were identical. They were both enriched in LGLs as determined by Percoll gradient fractionation and expressed the Leu-11b+, CD3~ phenotype as determined by cell sorting experiments (data not shown).

Table 1 HER2/neu-overexpressing ovarian cancer targets are resistant to lysis and conjugation by LAK cells and do not express ICAM-1 determinants

<table>
<thead>
<tr>
<th>Target</th>
<th>% lysis</th>
<th>% conjugation</th>
<th>ICAM-1 expression</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2/neu~ targets</td>
<td>87 ± 5</td>
<td>49 ± 6</td>
<td>27 ± 3</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>HER/neu~ targets</td>
<td>26 ± 3</td>
<td>16 ± 2</td>
<td>7 ± 1</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>SKOV-3</td>
<td>29 ± 4</td>
<td>21 ± 3</td>
<td>11 ± 2</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>436</td>
<td>29 ± 4</td>
<td>21 ± 3</td>
<td>11 ± 2</td>
<td>11 ± 3</td>
</tr>
</tbody>
</table>

* Cumulative data from five independent cytotoxicity assays which compared HER2/neu-overexpressing SKOV-3 and 436 targets to 6 HER2/neu~ targets (OV2CAR, 204, 222, CaOV-3, SRO-82, and PA-1). The data represent percentage specific lysis at three E:T ratios (mean ± SE). The average lysis of the five nonexpressing targets from each experiment was used to compute the mean ± SE shown.

You may refer to the table above for the detailed data. The table illustrates the resistance of HER2/neu-overexpressing targets to lysis and conjugation compared to nonexpressing targets.

IFN-γ Enhances the Sensitivity of HER2/neu-overexpressing Targets to LAK Lysis but Has no Effect on HER2/neu-nonexpressing Target Cells. The ability of IFN-γ to up-regulate ICAM-1 (6) and down-regulate HER2/neu expression (5) suggested it might be effective in enhancing the sensitivity of HER2/neu~ tumor cells to LAK lysis. Accordingly, overexpressing (436 and SKOV-3) and nonexpressing (SRO-82, 204, PA-1, 222) cell lines were exposed to 2000 units/ml IFN-γ for 3 days and tested as targets in a LAK cytotoxicity assay. Table 2 demonstrates a representative experiment where the viability (>95%) and the recovery (approximately 65%) when compared to cells cultured without IFN-γ following IFN-γ treatment were comparable between all targets irrespective of their HER2/neu phenotype. However, IFN-γ markedly increased the sensitivity of 436 and SKOV-3 targets to LAK lysis (approximately 3–5-fold) but had no effect on the sensitivity of the HER2/neu-nonexpressing cell lines (Table 2). This specific enhancement of cytotoxicity in only HER2/neu-expressing targets has been consistently detected in six experiments.

In some experiments, IFN-γ could enhance the susceptibility to lysis of the HER2/neu~ targets to levels which were comparable to those of HER2/neu~ cells (Fig. 1). However, in other experiments, IFN-γ only partially reversed resistance, with a resulting cytotoxicity curve that was considerably increased but still significantly lower than that of HER2/neu-nonexpressing targets simultaneously tested with the same effectors.

The enhancement of LAK cell lysis was somewhat dependent upon the concentration of IFN-γ used (Fig. 2). There was little effect from 2 and 20 units/ml, while 200 units/ml were optimal. There was no further enhancement when concentrations as high as 2000 units/ml were used. Therefore, the following experiments were performed using 200 units/ml IFN-γ.

Analysis of Cell Surface Molecules. In order to understand the mechanism(s) by which IFN-γ enhanced the sensitivity of HER2/neu-overexpressing targets, we studied its effects on the expression of three determinants (HLA class I, ICAM-1, and HER2/neu) which could potentially regulate sensitivity to LAK lysis. As shown in a representative experiment (Table 3), IFN-

Table 2 Effect of IFN-γ treatment on HER2/neu-non- and over-expressing ovarian cell lines

<table>
<thead>
<tr>
<th>Target</th>
<th>IFN-γ</th>
<th>% recovery</th>
<th>No. of LAKs required for 30% lysis (x10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2/neu~</td>
<td>-</td>
<td>100</td>
<td>2.5</td>
</tr>
<tr>
<td>SRO-82</td>
<td></td>
<td>57</td>
<td>2.5</td>
</tr>
<tr>
<td>204</td>
<td></td>
<td>100</td>
<td>2.0</td>
</tr>
<tr>
<td>204</td>
<td></td>
<td>65</td>
<td>2.5</td>
</tr>
<tr>
<td>222</td>
<td></td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>222</td>
<td></td>
<td>88</td>
<td>1.0</td>
</tr>
<tr>
<td>PA-1</td>
<td></td>
<td>100</td>
<td>2.0</td>
</tr>
<tr>
<td>PA-1</td>
<td></td>
<td>70</td>
<td>3.0</td>
</tr>
<tr>
<td>HER2/neu~</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKOV-3</td>
<td></td>
<td>100</td>
<td>17.0</td>
</tr>
<tr>
<td>SKOV-3</td>
<td></td>
<td>64</td>
<td>5.5*</td>
</tr>
<tr>
<td>436</td>
<td></td>
<td>100</td>
<td>50.0</td>
</tr>
<tr>
<td>436</td>
<td></td>
<td>63</td>
<td>10.0*</td>
</tr>
</tbody>
</table>

* 2000 units/ml for 3 days.

You may refer to the table above for detailed data. The table illustrates the effect of IFN-γ treatment on HER2/neu-over- and non-expressing ovarian cell lines. The percentage recovery and the number of LAKs required for 30% lysis are presented.

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IFN-γ-TREATED HER2/neu* CELLS AND CYTOTOXICITY

Fig. 1. Effect of IFN-γ on the sensitivity of SRO-82 (○) and SKOV-3 (●) to LAK cells. The targets were treated with (— — —) or without (— — —) 2000 units/ml IFN-γ for 3 days and were mixed with the LAKs for cytotoxicity assays. Points, specific lysis, mean of quadruplicate samples; bars, SD.

Fig. 2. Dose effect of IFN-γ on the sensitivity of SKOV-3 cells to LAK lysis. SKOV-3 cells were treated with 0 (●), 2 (○), 20 (△), 200 (▲), or 2000 (▼) units/ml IFN-γ for 3 days. Points, specific lysis, mean of three separate experiments; bars, SD.

γ treatment (200 units/ml, 3 days) of HER2/neu-overexpressing cells (SKOV-3 and 436) significantly induced ICAM-1 expression, modestly increased class I expression and decreased HER2/neu expression by approximately 60%. Experiments utilizing only 20 units/ml of IFN-γ produced less impressive changes (approximately 30% increase in class I and 35% decrease in HER2/neu expression), while 2000 units/ml were not any more effective than 200 units/ml (not shown). Similar treatment of HER2/neu-nonexpressing targets (SRO-82 and 204) modestly increased ICAM-1 expression and markedly increased class I expression.

Kinetics of IFN-γ Effect. The sensitivity of treated targets actually decreased after only 1 day of exposure to IFN-γ and was comparable to nontreated SKOV-3 cells at 2 days (Fig. 3). By 3 days of treatment, there was a consistent enhancement of lysis which did not further increase at 4 days (Fig. 3). The modulation of membrane antigen expression induced by IFN-γ also depended upon duration of treatment (Fig. 4). As shown, IFN-γ induced a progressive down-regulation of HER2/neu expression and up-regulation of class I expression with a maximum effect at day 3 (no further change was observed at day 4; data not shown). In contrast, the induction of ICAM-1 expression was maximal after only 1 day of IFN-γ treatment.

Reversibility of IFN-γ-induced Enhancement of Sensitivity. Since IFN-γ treatment decreased the recovery of the HER2/neu-overexpressing targets (Table 2), their increased sensitivity to lysis could be due to a selective survival of a subpopulation of cells that are inherently sensitive to lysis and overexpress ICAM-1 determinants. If this were the case, the LAK-sensitive phenotype should continue even after removal of IFN-γ. To study this hypothesis, SKOV-3 cells were treated for 3 days with or without 200 units/ml IFN-γ (t = 0) and then washed and reincubated in fresh media without IFN-γ for 24 h (t = 24 h). At each time point, they were tested as targets for LAK cytotoxicity (using different effector cell cultures) and for the expression of HER2/neu and ICAM-1. Within 24 h after reincubation in fresh media, the IFN-γ-induced enhancement

Table 3 Flow cytometric analysis of cell surface molecules after IFN-γ treatment*

<table>
<thead>
<tr>
<th>Target</th>
<th>HER2/neu</th>
<th>HLA class I</th>
<th>ICAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRO-82</td>
<td>—</td>
<td>73</td>
<td>45</td>
</tr>
<tr>
<td>SRO-82</td>
<td>+</td>
<td>1107</td>
<td>90</td>
</tr>
<tr>
<td>SKOV-3</td>
<td>—</td>
<td>1783</td>
<td>1705</td>
</tr>
<tr>
<td>SKOV-3</td>
<td>+</td>
<td>869</td>
<td>2714</td>
</tr>
<tr>
<td>436</td>
<td>—</td>
<td>1103</td>
<td>1180</td>
</tr>
<tr>
<td>436</td>
<td>+</td>
<td>564</td>
<td>2098</td>
</tr>
</tbody>
</table>

* Targets were treated with or without IFN-γ (200 units/ml for 3 days) and then assayed. The values express the mean channel fluorescence above the negative control (always <20) determined by fluorescence-activated cell sorter analysis. The monoclonal antibodies used in this study were c-erbB-2, W6/32, and CD54.
IFN-γ-TREATED HER2/neu+ CELLS AND CYTOTOXICITY

Fig. 4. Fluorescence-activated cell sorter analysis of antigen expression on SKOV-3 treated with or without IFN-γ for different periods of time. SKOV-3 cells were incubated with or without IFN-γ (200 units/ml) for 1, 2, or 3 days, and the expression of HER2/neu, ICAM-1, and HLA-1 was determined. . . . , negative control (no primary antibody); ----- untreated SKOV-3; --- IFN-γ-treated SKOV-3 (200 units/ml).

Fig. 5. Reversibility of IFN-γ-induced alterations in sensitivity to lysis and membrane antigen expression. Targets were treated with ( ) or without ( ) 200 units/ml IFN-γ for 3 days (time 0) and then washed and reincubated in fresh media without IFN-γ, for 24 h. Targets were then assayed for sensitivity to LAK cell lysis (top) and expression of HER2/neu and ICAM-1 (bottom). Results are expressed as percentage lysis, mean ± SE of quadruplicate samples. A second experiment gave identical results.

of lysis was reversed (Fig. 5). The reversibility of enhancement rules out the possibility of the selective survival (during IFN-γ treatment) of an inherently LAK-sensitive subpopulation and indicates that increased LAK cell lysis is due to the reversible effects of IFN-γ on tumor targets. The reversal of enhanced lysis correlated well with the reexpression of HER2/neu (Fig. 5). However, ICAM-1 expression was still markedly enhanced at 24 h after exposure to IFN-γ. In the experiment shown in Fig. 5, the LAK effector cells used at t = 24 h were considerably more potent than those used at t = 0. This explains why both IFN-treated and nontreated targets are lysed more efficiently at t = 24 h. The experiment was repeated using equipotent LAK populations at the two time points, and while the extra 24-h incubation in fresh media did not affect the lysis of the nontreated targets, it completely reversed the IFN-induced enhancement of treated targets. The resulting cytotoxicity curves (at 24 h) for both IFN-treated and nontreated cells were again identical.

IFN-γ Treatment of SKOV-3 Increases the Conjugation with LAK Cells. To test whether IFN-γ treatment affected HER2/neu-overexpressing targets in their initial interactions with LAKs, we utilized the single cell conjugation assay. Treatment of HER2/neu-overexpressing targets with IFN-γ (200 units/ml for 3 days) significantly (P < 0.05) increased their ability to be bound by LAK LGLs (percentage conjugation = 31 ± 2, mean ± SE of four independent experiments) as compared to nontreated targets (17 ± 1).

Effect of Anti-ICAM-1 Antibody on the Increased Sensitivity to Lysis. To test whether the IFN-γ-induced enhancement of lysis was due to the cytokine's ability to up-regulate ICAM-1 determinants, we performed the cytotoxicity assay in the presence of anti-ICAM-1 antibodies. The monoclonal antibody was from the 84H10 clone, which has been previously reported to be capable of blocking cell-mediated cytotoxicity (9). The monoclonal antibody was first dialyzed and then added to wells of the LAK cytotoxicity assay at a concentration of 10 μg/ml. A
Cytotoxicity assay was performed without antibodies (•) or with the addition of Targets were treated without (a) or with (b) IFN-γ (200 units/ml for 3 days). The present results. The experiment was repeated using 1 μg/ml of both antibodies and gave identical by anti-ICAM-1 antibody in b is statistically significant (P < 0.05) at E:T ratios of 20:1 and 10:1 when compared to groups assayed in the absence of antibody. The experiment was repeated using 1 μg/ml of both antibodies and gave identical results. similarly prepared isotype control monoclonal antibody (anti-MKL) was added in identical concentrations. Anti-ICAM-1 antibody had no effect on the LAK lysis of nontreated SKOV-3 cells (Fig. 6a). This result is consistent with the absence of ICAM-1 expression on these nontreated targets (Table 1). However, anti-ICAM-1 antibody significantly decreased the lysis of IFN-γ-treated SKOV-3 cells such that their sensitivity was comparable to that of the non-IFN-γ-treated targets (Fig. 6b). The isotype-identical control antibody (anti-MKL) had no effect (Fig. 6b).

DISCUSSION

We have previously reported (3) that HER2/neu-overexpressing ovarian tumor lines are relatively resistant to LAK lysis when compared to nonexpressing ovarian lines. The present study indicates that they also respond differently as targets when exposed to IFN-γ. Incubation with at least 200 units/ml for at least 3 days markedly enhanced their sensitivity to lysis, while the sensitivity of HER2/neu-nonexpressing targets was unaffected. In some experiments, LAK cell-mediated lysis of IFN-γ-treated HER2/neu-overexpressing targets was comparable to that of HER2/neu-nonexpressing ones (Fig. 1), suggesting a complete reversal of resistance. However, complete reversal was not consistently observed, and it is possible that there are other defects not corrected by IFN-γ which could also contribute to the relative LAK resistance of HER2/neu-overexpressing targets. Enhancement of lysis was not due to IFN-γ-induced selective survival of LAK-sensitive targets from a heterogeneous tumor cell population but was mediated by the cytokine's reversible effects on targets. Interferon markedly up-regulated the expression of ICAM-1 on HER2+ targets, and anti-ICAM-1 antibodies were capable of reversing the enhanced LAK lysis against these cells. These results indicate that the enhancement of LAK lysis is mediated by the induction of ICAM-1 on targets. However, some of our data are inconsistent with this hypothesis as the sole explanation for the results. First, although the up-regulation of ICAM-1 on HER2/neu-nonexpressing targets was less impressive than on HER2/neu-overexpressing targets, it was still quite significant (Table 3) but did not result in enhanced lysis. It is possible that there is a threshold level of ICAM-1 expression that must be reached for a given amount of lysis and that beyond this level there is little correlation with sensitivity to LAK cell lysis. Another possible explanation is that IFN-γ treatment affected some negative determinants of sensitivity on HER2/neu-nonexpressing cells. A good candidate would be its marked up-regulation of class I expression on these cells which was, in contrast, very modest on HER2/neu-overexpressing targets. In fact, we have found consistent decreases in the LAK cell lysis of HER2/neu-nonexpressing targets following their treatment with very high concentrations of IFN-γ (2000–4000 units/ml) for 3–4 days.

A second problem with the hypothesis that up-regulation of ICAM-1 is sufficient for enhanced lysis is the poor temporal correlation between the interferon-induced increase in lysis and ICAM-1 up-regulation on HER2/neu-overexpressing cells. Increased lysis required at least 3 days of exposure to IFN-γ, while maximal up-regulation of ICAM-1 was achieved within 24 h (Figs. 3 and 4). In addition, enhanced lysis was reversed within 24 h of removal of IFN-γ, but ICAM-1 expression was still markedly elevated (Fig. 5). It is possible that up-regulation of ICAM-1 must work in conjunction with a second IFN-γ-induced sensitizing event for enhanced lysis to occur. This second event may not occur until the third day of treatment and may be rapidly reversed within 24 h of removal of IFN-γ. These data further support the conclusion that, although induction of ICAM-1 expression is important in the enhanced lysis of IFN-γ-treated targets, it is not the only explanation.

It is also possible that the IFN-γ-induced down-regulation of HER2/neu plays a role in the enhanced lysis of HER2/neu-overexpressing targets. Although expression was decreased by only 60%, this may be sufficient to override any possible negative signals to LAK lysis. A role for down-regulation of HER2/neu could also explain the specificity of IFN-γ-induced enhancement (i.e., only in the HER2/neu-overexpressing targets) as well as the kinetics of enhancement and reversibility, since HER2/neu down-regulation was optimal after 3 days of treatment, the time at which enhanced lysis was first detected and was reversed by 24 h of reincubation in IFN-γ-free media.

We currently have no adequate explanation for the protective effect of IFN-γ seen after 24 h of treatment (Fig. 3). Although up-regulation of class I expression may induce protection against non-major histocompatibility complex-restricted cytotoxicity (reviewed in Ref. 10), this is probably not the cause of the early (24 h) detected protection, since class I up-regulation by IFN was only significant after 48 and 72 h of treatment. The role of ICAM-1 as a cell adhesion molecule as well as the IFN-induced enhancement of effector-target cell binding suggests that at least part of the increased lysis of HER2+ targets is due to an enhancement of the initial recognition stages of the effector-target interaction. However, preliminary cold target inhibition studies have been inconclusive. It is thus still possible that the IFN-induced enhanced expression of ICAM-1 facilitates target lysis by affecting postbinding events.

A recent report (6) showed a similar ability of IFN-γ to increase the sensitivity of neuroblastoma cells to LAK cell lysis and induce the expression of ICAM-1. The temporal correlation between induced expression and enhanced sensitivity in that study was even better than in ours and, in addition, anti-ICAM-1 antibodies blocked the increased LAK cell lysis. However, blocking by anti-ICAM-1 was not complete, and the authors...
concluded that, similar to our study, induction of ICAM-1 determinants only partially explains enhanced lysis. In contrast, other studies (11) have shown a lack of effect of IFN-γ on target cell sensitivity to LAK cells, while lysis by tumor-infiltrating T-lymphocytes was enhanced. In fact, other studies (12–14) reported that exposure to IFN-γ depresses sensitivity to natural killer/LAK cells, presumably through its ability to increase class I expression. One explanation for these inconsistencies may be differences in the levels of ICAM-1 and class I and in the sensitivity to natural killer/LAK cells in the non-IFN-γ treated cells. A natural killer/LAK-resistant target cell with high class I expression but minimal ICAM-1 expression would be more likely to respond to IFN-γ with enhanced sensitivity to lysis, since ICAM-1 up-regulation would have more profound effects than modest class I up-regulation. The phenotype of HER2/neu"-expressing ovarian cancer cells fits this profile.

Our results may have clinical therapeutic relevance. Immunotherapy has been used with minimal success on ovarian cancers (4). The resistance of HER2/neu-overexpressing cells to tumor necrosis factor (2) and LAK cells suggests that these cancers will be particularly difficult to treat with immunotherapy. In addition, this subset of ovarian tumors is the most aggressive and is associated with the poorest prognosis (1). The ability of IFN-γ to sensitize these cells to lymphocyte cytotoxicity, in addition to the direct antiproliferative effect of IFN-γ (especially on HER2/neu" ovarian cancer cells; Ref. 5), suggests that IFN-γ used in conjunction with other biological agents could have a beneficial antitumor effect on patients.

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REFERENCES


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