Differential Sensitivity to Non-Major Histocompatibility Complex-restricted Recombinant Interleukin 2-activated Lymphocyte Killing of Human Mammary Epithelial MCF-10A Cells Overexpressing Oncogenes or Protein Kinase A Subunits

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ABSTRACT

The sensitivity of human tumor cells to activated lymphocytes is considered to play an essential role in the antitumor activity of recombinant interleukin-2 (rIL-2)-based immunotherapy. We have investigated the effects of several genes involved in the regulation of cell growth and transformation on the sensitivity of human mammary epithelial MCF-10A cells to non-MHC-restricted, rIL-2-activated lymphocytes. Therefore, the lysability of MCF-10A cells overexpressing activated oncogenes (Ha-ras, erbB-2, and a mutated p53), growth factors [transforming growth factor α (TGFα)], or cAMP-dependent protein kinase A subunits (RIOα, RIβ, and Coα) was evaluated comparatively at different effector:target ratios by a 51Cr release assay. Parental MCF-10A, MCF-10A p53-mutated, and MCF-10A RIβ cells showed an intermediate sensitivity. Lysability was increased significantly in MCF-10A Ha-ras, MCF-10A TGFα, and MCF-10A RIOα cells, reduced in MCF-10A Coα cells, and completely abrogated in MCF-10A erbB-2 cells. These differences could not be explained by simple changes in the cell surface expression of MHC class I and intercellular adhesion molecule-1 proteins or by secretion of TGFβ. Treatment with TAb 250, a mouse anti-p185erbB-2 monoclonal antibody, or down-regulation of p185erbB-2 expression resulted in circumvention of MCF-10A erbB-2 cell resistance.

We conclude that molecular changes at the single-gene level resulting in alterations of intracellular signaling and/or cell transformation modulate sensitivity of human mammary epithelial cells to non-MHC-restricted, rIL-2-induced cytotoxicity, regardless of MHC class I and/or intercellular adhesion molecule-1 expression or TGFβ secretion. Furthermore, anti-p185erbB-2 monoclonal antibodies may be useful as adjuncts to rIL-2 treatment in patients with erbB-2-overexpressing tumors.

INTRODUCTION

Non-MHC-restricted cytotoxic lymphocytes play an important role in the surveillance against tumor development (1). NK3 cells, which represent 5–15% of circulating lymphocytes, are capable of killing several tumor cell targets without previous activation and in a non-MHC-restricted fashion. (1). LAK activity is generated on PBLs by IL-2 treatment. These lymphocytes, after exposure to rIL-2, acquire the capacity of killing autologous and allogeneic tumor cells. LAK cytotoxicity is non-MHC restricted, resides mostly in NK cells, and can be exerted also against tumor cell targets not sensitive to spontaneous NK cytotoxicity. LAK activity can be generated and transferred adoptively to cancer patients by rIL-2 administration (2).

Cultured human tumor cells are heterogeneous in their in vitro sensitivity to cell-mediated cytotoxicity and specifically to NK and LAK effectors. Such a difference has been ascribed to various cancer cell phenotypes, including the degree of differentiation (3), the expression of adhesion molecules (4, 5) and MHC class I antigens (6, 7), and the production of immunodepressing factors such as TGFβ (8, 9).

It is a common finding that molecular changes leading to cell transformation induce an increased sensitivity to non-MHC-restricted cytotoxicity. Oncogene expression has been correlated to changes in NK and LAK sensitivity. In this respect, v-Ki-ras transformation increases the sensitivity of Rat-1 fibroblasts to NK cells (10). Expression of an activated, point-mutated c-Ha-ras oncogene is capable of enhancing the susceptibility of mouse C3H 10T1/2 cells to NK cytolysis (11). On the other hand, it has been shown that transfection of human colon car-

Received 5/15/95; revised 8/11/95; accepted 8/15/95.

1 Supported by the Italian Association for Cancer Research; Consiglio Nazionale della Ricerca, Progetto Finalizzato: Applicazioni Cliniche della Ricerca Oncologica; the Elsa U. Pardee Foundation; and NIH Grant CA16221-01A1. M. C. and A. R. are recipients of fellowships from the Italian Association for Cancer Research.

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The abbreviations used are: NK, natural killer; TGF, transforming growth factor; PKA, protein kinase A; MAb, monoclonal antibody; PBL, peripheral blood lymphocyte; ICAM-1, intercellular adhesion molecule-1; LAK, lymphokine-activated killing; rIL-2, recombinant interleukin-2; EGF, epidermal growth factor; MT-1, metallothionein-1; mut, mutated; FBS, fetal bovine serum; IU, international unit; FACS, fluorescence-activated cell sorting.

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cinoma cells with a c-Ha-ras oncogene leads to the acquisition of NK resistance (12). Expression of c-erbB-2 correlates generally with resistance to LAK in human ovarian tumor cell lines (13). This information has been derived generally from studies in which rodent cell systems or human tumor cell lines have been used. Taking into account the complexity of genetic events causing human carcinogenesis, it is difficult to obtain useful information on the specific role of single oncogenes in the determination of lymphocyte sensitivity by comparative studies on human cultured tumor cell lines. Furthermore, rodent cell systems are derived from phenotypically unstable fibroblast cell lines, such as NIH-3T3 cells, which could be transformed easily by a chemical agent or by a single activated oncogene. These systems have been used successfully for the identification of activated oncogenes by transfection assays. However, they are generally of limited value for the study of phenotypical changes correlated to the modifications in the sensitivity to activated lymphocytes. In this regard, we have taken advantage of the recent establishment of MCF-10A cells, which have unique characteristics of nontransformed, spontaneously immortalized, human mammary epithelial cells (14). We developed several MCF-10A clones transformed following transfection or infection with appropriate expression vectors containing activated oncogenes, such as Ha-ras, mutated tumor suppressor genes, such as a mutated p53, growth factor genes, such as TGFα, and growth factor receptor genes, such as erbB-2 (15–17). Furthermore, we have obtained MCF-10A clones that overexpress the various PKA genes recently (18). This provides a useful in vitro model system for studying the mechanisms of human mammary epithelial cell transformation.

In this study, we have evaluated the sensitivity of various MCF-10A derivatives to rIL-2-activated human lymphocytes in a 51Cr release cytotoxicity assay. A definite pattern of sensitivity to rIL-2-activated lymphocytes was observed. Therefore, we have investigated whether changes in the expression of surface molecules, such as MHC class I and ICAM-1, or of secreted factors, such as TGFβ, could account for the differences in the sensitivity to rIL-2-activated lymphocytes observed in the various MCF-10A cell lines. Because the highest degree of resistance was observed in MCF-10A erbB-2, we have evaluated whether treatment with an anti-p185erbB-2 mouse MAB or p185erbB-2 down-regulation could increase the sensitivity of these cells to activated lymphocytes.

MATERIALS AND METHODS

Cell Cultures. MCF-10A cells and their derivatives were grown in a 1:1 (v/v) DMEM and Ham’s F12 medium (Flow Laboratories, Milan, Italy) mixture supplemented with 5% heat-inactivated horse serum, 0.5 μg/ml hydrocortisone, 10 ng/ml EGF, and 10 μg/ml insulin. MCF-10A Ha-ras cells were generated by cotransfection of MCF-10A cells with an expression vector plasmid containing the human activated c-Ha-ras proto-oncogene and an expression vector plasmid containing the neomycin resistance gene (15). MCF-10A neo cells are MCF-10A cells containing only the neomycin resistance gene. MCF-10A TGFα, MCF-10A Ca, MCF-10A Rκ, and MCF-10A R1β cells were obtained by infection of MCF-10A cells with amphotropic retroviral vectors containing the neomycin resistance gene with the human TGFα, Ca, Rκ, and R1β genes (15, 18). MCF-10A erbB-2 cells were generated following infection of MCF-10A cells with an amphotropic retroviral vector containing the hygromycin resistance gene with the human c-erbB-2 proto-oncogene (16). Expression of these genes was under the transcriptional control of the heavy metal-inducible mouse MT-1 promoter. Therefore, MCF-10A TGFα, MCF-10A Ca, MCF-10A Rκ, MCF-10A R1β, and MCF-10A erbB-2 cells were grown continuously in the presence of 1 μM CdCl2 to induce the expression of the MT-1 promoter maximally. We have shown previously that this concentration of CdCl2 is not toxic for MCF-10A cells and does not affect their growth (15). MCF-10A p53 mutant cells were generated by transfection of MCF-10A cells with an expression vector plasmid containing the p53 gene with a human-mutated p53 gene containing a point mutation at codon 238 with a cysteine-to-phenylalanine substitution, as described previously (17). The expression of each transduced gene in the various MCF-10A cell derivatives was assessed properly (15–17). The human tumor cell line SKOV-3 was obtained from the American Type Culture Collection (Rockville, MD; cell line HTB77) and was grown in Iscove’s DMEM containing 10% FBS and 2 mM l-glutamine (19). All cell lines were maintained in a humidified incubator with 5% CO2 at 37°C.

Cell-mediated Cytotoxicity. The sensitivity of MCF-10A-derived cell lines by LAK cells was evaluated in a 51Cr release cytotoxicity assay at different effector:target ratios in complete medium containing heat-inactivated 10% FBS. Target cells were seeded in 96-multiwell plates (Becton Dickinson, Lincoln Park, NJ) and incubated with 51Cr (37 megabecquerels, 1.00 mCi; Amersham, Buckinghamshire, United Kingdom; 30,000–50,000 cpm/well) for 30 min at 37°C. PBLs obtained by ficoll-hypaque gradient separation were incubated in RPMI 1640 medium with 10% FBS, l-glutamine, and antibiotics. rIL-2 (1,000 IU/ml; Cetus Corp., Emeryville, CA) was added subsequently, and the PBLs were incubated at 37°C for 18 h. IL-2 treatment of human PBLs, under these conditions, induces LAK activity, which seems to be mostly due to activated NK cells (20). Parallel experiments were conducted with LAK cells generated by exposure to 100 IU rIL-2 for 5 days. After washing with PBS without Ca2+ and Mg2+ LAK cells were added to the dishes at different effector:target ratios. Target cells and immune effectors were incubated at 37°C for 4 h. One hundred μl supernatant medium from each of triplicate samples were counted in a gamma counter (Beckman Instruments, Fullerton, CA). Results were expressed as percentage specific release:

\[
\text{specific release} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100
\]

using 51Cr in supernatant from the experimental incubation, spontaneous 51Cr release from target cells without lymphocytic effectors, and total 51Cr from the controls. The SD of quadruplicate counts was <10%. Results from the cytotoxicity experiments were also expressed as lytic units/106 lymphocytes (1 lytic unit was defined as the number of cells required for obtaining 30% cell lysis). For the evaluation of anti-p185erbB-2 antibody effects on LAK sensitivity of MCF-10A erbB-2 cells,
these cells were treated for 30 min and 6, 12, and 24 h prior to the 51Cr release cytotoxicity assay with 1 μg/ml TAb 250 MAb. As a control, MCF-10A erbB-2 cells were exposed 24 h to the anti-EGF-R 528 MAb [a gift from Dr. John Mendelsohn (Memorial Sloan-Kettering Cancer Center, New York, NY)] or to the control mouse IgG1 (Sigma Chemical Co., St. Louis, MO).

FACS Analysis. Cells were detached with a nonenzymatic cell dissociation solution (Sigma) and incubated for 30 min at 4°C with the mouse antibody human leukocyte antigen-avidin-biotin complex (DAKO Corp., Glostrup, Denmark) directed against human MHC class I antigens. After two washes with PBS (Flow Laboratories) containing 0.5% BSA, cells were incubated with FITC-labeled goat antimouse serum (DAKO) at a 1:20 dilution for 30 min at 4°C. Flow cytometric analysis was performed with a FACSscan (Becton Dickinson, San Jose, CA). Forward and side-scatter cell gating was performed to detect fluorescence only on intact living cells. Histograms showing the specific immunofluorescent labeling were generated using the Consort 30 software (Becton Dickinson). Similarly, ICAM-1 expression was evaluated by indirect immunofluorescence and FACS analysis by incubating the cells for 30 min at 4°C with the CL203.4 MAb [kindly provided by Dr. Soldano Ferrone (New York Medical College, Valhalla, NY)].

TGFβ Production. Production of TGFβ was evaluated in the conditioned medium collected from cultures of MCF-10A-derived cell lines as competing binding activity against 125I-labeled TGF-β (New England Nuclear Research Products, Wilmington, DE) on AKR2B cells and expressed as ng/10⁶ cells for 48 h. Such values were extrapolated by a control displacement curve, which was calculated by using known amounts of unlabeled TGFβ, as described previously (21).

Competition Assay. TAb 250 was radiolabeled with 125I (Amersham, Arlington Heights, IL) to a specific activity of 2–5 μCi/μg as described previously (22). SKOV-3- and MCF-10A-derived cell lines were harvested in Ca²⁺- and Mg²⁺-free PBS containing 2 mM EDTA and 1% glucose. Cells were washed once in PBS and resuspended in binding buffer containing DMEM, 0.1% BSA, and 50 mM HEPES (pH 7.4; Whittaker Bioproducts, Walkersville, MD). Cells (1 × 10⁴/tube) were incubated with 10 μl labeled TAb 250 (8000 cpm/tube) alone or in combination with 10 μl varying concentrations of cold TAb 250 at 4°C for 4 h on a rotary shaker. The percentage of binding, dissociation constant, and p185erbB-2 binding sites/cell were determined as described previously (19).

RESULTS

Sensitivity of the MCF-10A Cell Lines to rIL-2-activated Lymphocytes. The sensitivity of MCF-10A cells to cytotoxic lymphocytes activated by rIL-2 treatment was evaluated by a 51Cr release cytotoxicity assay. The lysability of MCF-10A cells transfected with an expression vector plasmid containing the neomycin resistance gene (MCF-10A neo cells) to activated human lymphocytes was similar to that of parental MCF-10A cells (data not shown). Therefore, MCF-10A neo cells were selected as the control cell line in the subsequent experiments. MCF-10A derivatives exhibited four different levels of sensitivity to rIL-2-activated cytotoxic lymphocytes (Fig. 1). The highest level of sensitivity was observed in MCF-10A neo cells, followed by MCF-10A TGFα, MCF-10A p53 mut, MCF-10A Ha-ras, and MCF-10A Ha-ras cells. Each value is the average of triplicate determinations of at least three different experiments.

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

**Fig. 1** Sensitivity of MCF-10A-derived cell lines to non-MHC-restricted, rIL-2-activated lymphocytes evaluated by a 51Cr release cytotoxicity assay at different effector:target ratios. MCF-10A cells were exposed for 4 h to PBL activated by 18-h treatment with 1000 IU/ml rIL-2. Lymphocyte cytotoxicity is expressed in C as lytic units/10⁶ effector cells: MCF-10A neo (1); MCF-10A erbB-2 (2); MCF-10A Ha-ras (3); MCF-10A p53 mut (4); MCF-10A RIIβ (5); MCF-10A TGFα (6); MCF-10A RIIα (7), and MCF-10A Ha-ras (8) cells. Each value is the average of triplicate determinations of at least three different experiments.
Oncogenes and PKA Subunits Modulate LAK Sensitivity

MCF-10A neo, MCF-10A Ha-ras, MCF-10A TGFα, MCF-10A erbB2, MCF-10A p53 mut, MCF-10A RIIα, and MCF-10A RIIβ cells, respectively. Each value is the average of triplicate determinations of at least three different experiments. Bars, SD.

Ha-ras, MCF-10A TGFα, and MCF-10A RIIβ cells (mean values, 250, 111, and 156 lytic units/10^6 effectors, respectively). MCF-10A neo, MCF-10A p53 mut, and MCF-10A RIIβ cells showed an intermediate sensitivity to activated lymphocytes (mean values, 53, 47, and 67 lytic units/10^6 effectors, respectively), whereas MCF-10A RIIα cells were less sensitive (mean values, 22 lytic units/10^6 effectors), and MCF-10A erbB-2 cells were completely resistant. Activation of lymphocytes by 5 days exposure to rIL-2 did not modify the pattern of MCF-10A cell sensitivity (data not shown).

Surface Expression of MHC Class I and ICAM-1 Molecules in the MCF-10A Cell Lines. We evaluated next whether the observed differences in MCF-10A cell sensitivity to activated lymphocytes could be correlated to changes in the expression of MHC class I and ICAM-1 proteins. In fact, these molecules are involved in the determination of tumor cell sensitivity to cytotoxic lymphocytes. MHC class I interaction with tumor antigens is essential for the recognition of cancer cells by MHC-restricted cytotoxic effectors (23). However, the influence of MHC class I in NK- and LAK-mediated tumor cell cytotoxicity is still unclear. According to the “missing self” hypothesis formulated by Ljunggren and Karre (7), a specific function of NK cells is to recognize and eliminate cells failing to express self-MHC class-I antigens. All MCF-10A cell derivatives, with the exception of only MCF-10A p53 mut cells, exhibited a reduced density of surface MHC class I compared with MCF-10A neo cells (Fig. 2). Therefore, it is possible to assume that in the MCF-10A cell system, the sensitivity to rIL-2-activated, non-MHC-restricted cytotoxicity is not correlated to the expression of MHC class I antigens.

It is known that several adhesion molecules are involved in the contact and recognition of tumor cell targets by MHC class
MCF-10A-derived cell lines were analyzed for cell surface expression of ICAM-1 by indirect immunofluorescence FACS analysis with the anti-ICAM-1 CL 203.4 antibody (outlined histograms). An isotypic, nonimmune mouse IgG was used as a negative control (black-filled histograms). Percentage of positive cells and mean fluorescence intensity have been represented as vertical bars: 1, MCF-10A neo; 2, MCF-10A Ha-ras; 3, MCF-10A TGFα; 4, MCF-10A erbB-2; 5, MCF-10A p53 mut; 6, MCF-10A RIa; 7, MCF-10A Co; and 8, MCF-10A RIIβ cells. Each value is the average of triplicate determinations of at least three different experiments. Bars, SD.

I-restricted and -nonrestricted lymphocytes. Costimulatory signals are generated in T cells after interaction of leukocyte function-associated antigen-1, an αβ nonpolymorphic, heterodimeric integrin receptor, with ICAM-1, an integral membrane glycoprotein belonging to the immunoglobulin superfamily, which is expressed by antigen-presenting cells or target cells (24). ICAM-1 expression has been considered to play a role also in the non-MHC-restricted cytotoxicity of human tumor cell targets (4, 5). Therefore, we have evaluated the expression of ICAM-1 on the various MCF-10A cell derivatives (Fig. 3). Cell surface ICAM-1 protein was almost unexpressed in MCF-10A neo and p53 mut cells, whereas it was expressed at high levels in MCF-10A Ha-ras and MCF-10A TGFα cells and at low levels in all the other MCF-10A clones. Therefore, the marked heterogeneity of ICAM-1 expression by MCF-10A cell lines failed to result in a pattern consistent with the different sensitivity to cytotoxic lymphocytes.

TGFβ Production by the MCF-10A Cell Lines. The production of immunodepressing factors could account for the differences in sensitivity to cytotoxic lymphocytes observed in the MCF-10A cell lines. In fact, it has been described that TGFβ depresses NK activity in PBLs and inhibits LAK generation (8, 9). Production of TGFβ was enhanced significantly in the majority of MCF-10A cell lines, with the exception of MCF-10A RIβ and MCF-10A Co cells, compared with MCF-10A neo cells (Fig. 4). An approximately 6-fold increase in TGFβ production was found in the LAK-resistant MCF-10A erbB-2 cells. However, high levels of TGFβ secretion in the conditioned medium were detected also in the LAK-sensitive MCF-10A cell derivatives, including MCF-10A Ha-ras and MCF-10A TGF-α.
TGF-β plays a major role in the sensitivity of MCF-10A cells to LAK binding sites on MCF-10A binding was detectable on MCF-10A neo, MCF-10A TGFe, MCF-10A Ca, MCF-10A Ras, MCF-10A RIIβ, MCF-10A p53 mut, or MCF-10A erbB-2 cells in which CdCl₂ was withdrawn for 7 days of cell culture.

Sensitivity of MCF-10A erbB-2 Cells to LAK after Anti-p185^{erbB-2} Tab 250 MAb Treatment and Down-Modulation of p185^{erbB-2} Expression. MCF-10A erbB-2 cells were not lysed by cytotoxic lymphocytes. Therefore, we have evaluated whether the interaction with the erbB-2 signaling or the down-regulation of p185^{erbB-2} expression could increase cell sensitivity to rIL-2-activated immune effectors. MCF-10A erbB-2 cells were treated with 1 μg/ml Tab 250 MAb. This antibody binds to the extracellular domain of p185^{erbB-2} and has agonistic properties, because it induces receptor autophosphorylation and down-regulation of p185^{erbB-2} expression in MCF-10A erbB-2 cells (data not shown), stimulates phospholipase C-γ1, and causes changes in cellular localization of specific PKC isozymes (25–27). We have evaluated Tab 250 MAb binding on MCF-10A-derived cell lines, and we have found about 8.16 × 10⁵ binding sites on MCF-10A erbB-2 cells, whereas no specific binding was detectable on MCF-10A neo, MCF-10A ras, MCF-10A TGFα, MCF-10A Ca, MCF-10A RIIα, MCF-10A RIIβ, and MCF-10A p53 mut cells (Table 1). LAK sensitivity of MCF-10A erbB-2 cells was enhanced significantly by 24 h treatment with Tab 250, whereas it was not modified by treatment with a MAb binding to a related but different peptide growth factor receptor, such as the anti-EGF-R MAb 528 or an unrelated IgG1 (Fig. 5). Moreover, Tab 250 did not mediate antibody-dependent cell cytotoxicity in these cells (data not shown). Because expression of the c-erbB-2 gene in the MCF-10A erbB-2 cells is under the transcriptional control of the CdCl₂-inducible mouse MT-1 promoter (16), we have evaluated the sensitivity to cytotoxic lymphocytes of MCF-10A erbB-2 cells grown in the absence of CdCl₂. MCF-10A erbB-2 cells cultured for 7 days in CdCl₂-deficient medium exhibited p185^{erbB-2} levels that were comparable to those of MCF-10A neo cells (Table 1) and resumed a sensitivity to rIL-2-activated cytotoxic lymphocytes similar to that observed in MCF-10A neo cells.

**DISCUSSION**

Experimental and clinical findings have led recently to the hypothesis that the failure of rIL-2-based immunotherapy to produce antitumor effects even in the presence of a clearly detectable in vivo immunomodulatory activity may be determined by tumor-specific events, such as the selection of cancer cell populations resistant to activated cytolytic effectors (28). However, the molecular mechanisms regulating the susceptibility of human tumor cells to natural immunity are still poorly understood. In fact, non-MHC-restricted cytotoxic lymphocytes are considered important antitumor effectors in the rIL-2-based immunotherapy of human cancer (2). Activated oncogenes have been described to modulate the sensitivity to NK and LAK effectors (10–13). Such studies, in which transfected rodent cells or human cultured tumor cell lines have been used generally, are, however, weakened by the genetic instability of undifferentiated rodent fibroblasts and by the phenotypical heterogeneity of cultured human tumor cell lines.

In this report, we have studied the relevance of single genes involved in the control of cell growth and transformation and in cellular signaling on the susceptibility of human cells to natural cytotoxicity. A novel experimental approach has been derived by the use of the human nontransformed, near-diploid mammary epithelial cell line MCF-10A. These cells have been induced to overexpress activated oncogenes, growth factors, or PKA subunits by retroviral gene transfer or by transfection with expression vector plasmids. We have found that these genes are capable of modulating the sensitivity of MCF-10A human mammary epithelial cells to rIL-2-induced, non-MHC-restricted cytotoxicity. Notably, overexpression of an activated c-Ha-ras proto-oncogene, which has been found generally to enhance the sensitivity to NK effectors (11), induces a higher susceptibility to rIL-2-activated lymphocytes. Similarly, overexpression of TGFα or the RIIα regulatory subunit of type I PKA confers a higher degree of sensitivity to rIL-2-activated lymphocytes. These results suggest that the RIIα, Ha-ras, and TGFα genes may act through a common pathway(s) to enhance the sensitivity of MCF-10A cells to rIL-2-activated lymphocytes. In this regard, we have demonstrated previously that expression and

**Table 1**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Dissociation constant</th>
<th>Binding sites/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKOV-3</td>
<td>4.35 × 10⁻¹⁰</td>
<td>18.5 × 10⁴</td>
</tr>
<tr>
<td>MCF-10A erbB-2</td>
<td>3.75 × 10⁻¹⁰</td>
<td>8.16 × 10⁴</td>
</tr>
</tbody>
</table>

*No specific binding for Tab 250 MAb was found in MCF-10A neo, MCF-10A ras, MCF-10A TGFα, MCF-10A Ca, MCF-10A Ras, MCF-10A RIIβ, MCF-10A p53 mut, or MCF-10A erbB-2 cells in which CdCl₂ was withdrawn for 7 days of cell culture.
activity of PKAI and Rlα is involved functionally in the process of cell transformation by TGFα or ras genes (18, 29, 30). c-erbB-2 overexpression has been correlated to LAK resistance in human ovary and breast cancer cell lines (13, 31). Similarly, overexpression of p185\(^{\text{erbB-2}}\) in MCF-10A cells leads to a loss of sensitivity to cytotoxic lymphocytes.

It has been proposed that MHC antigens and adhesion molecules might be involved in the determination of tumor cell sensitivity to NK and LAK cells (4–7). Therefore, we have evaluated whether changes in MHC class I and ICAM-1 expression could account for the differences in sensitivity to cytotoxic lymphocytes in the MCF-10A cell system. The various MCF-10A cell lines were heterogeneous in the cell surface expression of these antigens. However, these changes did not correlate with the differences in the sensitivity to LAK. In fact, MHC class I antigen expression is reduced in almost all the MCF-10A-derived cell lines, with the exception of only MCF-10A p53 mut, which displays the same sensitivity to MCF-10A neo to rIL-2-activated lymphocytes. MCF-10A-derived cell lines were also capable of secreting TGFβ, which is a powerful inhibitor of NK and LAK function (8, 9). Although secreted TGFβ activity was ≈5-fold higher in MCF-10A erbB-2 cells than in MCF-10A neo cells, as measured in a 125I-labeled TGF-β radioreceptor assay, it was elevated also in the LAK-sensitive MCF-10A TGFα, MCF-10A Ha-ras, and MCF-10A Rlα cells. Finally, we have addressed whether the resistance of MCF-10A erbB-2 cells to rIL-2-activated cytotoxic lymphocytes could be affected by interference with c-erbB-2 proto-oncogene intracellular signaling or with p185\(^{\text{erbB-2}}\) protein expression. A 24-h exposure of MCF-10A erbB-2 cells to 1 μg/ml TAb 250 MAb, which has ligand-mimicking properties, reconstitutes the sensitivity of these cells to cytotoxic lymphocytes to a degree comparable to that observed in control MCF-10A neo cells. Notably, at the same experimental conditions, the anti-EGF-R 528 MAb and an unrelated IgG1 were unable to reconstitute sensitivity to the cytotoxic lymphocytes. Moreover, CdCl₂ withdrawal, which determines the reduction of p185\(^{\text{erbB-2}}\) protein expression in MCF-10A erbB-2 cells, enhances their sensitivity to LAK. These results are in agreement with those of Fady et al. (31), who reported that sensitivity to LAK-mediated cytotoxicity is enhanced selectively by IFN-γ treatment in human tumor cell lines overexpressing p185\(^{\text{erbB-2}}\). This effect was correlated to down-modulation of cell membrane expression of p185\(^{\text{erbB-2}}\) (31). Furthermore, induction of ICAM-1 expression in tumor cells is necessary but not sufficient for the enhancement of sensitivity to LAK lysis by IFN-γ (31).

The differences in sensitivity to non-MHC-restricted, rIL-2 activated lymphocytes observed in the various MCF-10A-derived cell lines are not related directly to differences in the surface expression of ICAM-1 and MHC class I antigens or in TGF-β production. Moreover, the LAK resistance of p185\(^{\text{erbB-2}}\)-overexpressing MCF-10A cells can be reverted by an anti-p185\(^{\text{erbB-2}}\) MAb. We suggest that novel methods for a proper selection of sensitive tumors and novel strategies for circumvention of resistance to immunotherapy could be derived by modulation of the expression of single genes involved in the regulation of cell sensitivity to cytotoxic lymphocytes. On the basis of these results, we propose that anti-p185\(^{\text{erbB-2}}\) MABs, with antitumor growth inhibitory properties and agonist-like activity, such as TAb 250 (25–27, 32), could be used in combination with rIL-2 in the treatment of human cancers with enhanced c-erbB-2 proto-oncogene expression.

REFERENCES


Fig. 5  Sensitivity of MCF-10A erbB-2 cells to 40:1 effector:target ratio after exposure to anti-p185\(^{\text{erbB-2}}\) TAb 250 MAb or down-regulation of surface p185\(^{\text{erbB-2}}\) induced by CdCl₂ deprivation. 1, MCF-10A erbB-2, untreated; 2–5, MCF-10A erbB-2 treated with 1 μg/ml TAb 250 for 30 min and 6, 12, and 24 h, respectively; 6, MCF-10A erbB-2 treated with unrelated IgG1; 7, MCF-10A erbB-2 treated for 24 h with 1 μg/ml anti-EGF-R MAb 528; and 8, MCF-10A erbB-2 cultured for 7 days in CdCl₂-deficient medium. Each value is the average of triplicate determinations from at least three different experiments. SEs were always less than 10%.


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