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# Modulation of the antigenic phenotype of human breast carcinoma cells by modifiers of protein kinase C activity and recombinant human interferons

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**Summary.** In the present study we have analyzed the effect of a synthetic protein kinase C (PKC) activator 3-(*N*-acetylamino)-5-(*N*-decyl-*N*-methylamino)-benzyl alcohol (ADMB) and the natural PKC-activating tumor-promoting agents 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and mezerein on the antigenic phenotype of T47D human breast carcinoma cells. All three agents increased the surface expression of the tumor-associated antigen BCA 225 and various cellular antigens, including HLA class II antigens, intercellular adhesion molecule 1 (ICAM-1) and c-*erb*B-2. Expression of the same antigens was also up-regulated to various extents in T47D cells by recombinant fibroblast (IFNβ) and immune (IFNγ) interferon. Shedding of BCA 225 from T47D cells was induced by TPA, mezerein, IFNβ and IFNγ, whereas ADMB did not display this activity. The ability of ADMB, TPA and mezerein to modulate the antigenic phenotype of T47D cells appears to involve a PKC-mediated pathway, since the PKC inhibitor, H-7, eliminates antigenic modulation. In contrast, the ability of IFNβ and IFNγ to enhance the synthesis, expression and shedding of BCA 225, as well as to enhance HLA class II antigens, c-*erb*B-2 and ICAM-1 expression, was either unchanged or modestly reduced by simultaneous exposure to H-7. Analysis of steady-state mRNA levels for HLA class I antigens, HLA class II-DRβ antigen, ICAM-1 and c-*erb*B-2 indicated that the ability of H-7 to inhibit expression of these antigens in TPA-, mezerein- and ADMB-treated cells was not a consequence of a reduction in the steady-state levels of mRNAs for these antigens. The results of the present investigation indicate that the biochemical pathways mediating enhanced antigenic expression in T47D cells induced by TPA, mezerein and the synthetic PKC activator ADMB are different from those induced by recombinant interferons. Furthermore, up-regulation of antigenic expression in T47D cells can occur by a PKC-dependent or a PKC-independent pathway.

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## Introduction

The expression of both histocompatibility antigens and tumor-associated antigens (TAA) on the surface of tumor cells can be augmented by treatment with bioresponse modulators, such as interferon and tumor necrosis factor α, and phorbol ester tumor promoters, such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA) [1, 13, 14, 17, 18, 20, 21, 26, 27]. Up-regulation of additional cellular antigens can be induced to a similar extent in both normal and tumor cells by bioresponse modulators indicating that this effect is a general property of these compounds and not restricted to TAA or cells of a specific histotype (for review see [1, 23]. For example, various interferons have been shown to enhance the expression of histocompatibility antigens, cellular antigens and TAA in breast carcinoma, central nervous system tumors, colon carcinoma and melanoma cells [20, 21, 23, 26, 27, 31, 38]. In addition, when administered to animals containing human tumor xenografts, recombinant human interferon augments the ability of excised tumors to bind monoclonal antibodies (mAb) specific for TAA [13, 23, 36, 42]. The use of bioresponse modifiers for increasing the expression of TAA by tumor cells may prove useful in reducing the antigenic heterogeneity in tumors in vivo and augmenting the ability of mAb to bind to tumors (for review see [1, 21, 25, 28].

TPA and recombinant human leukocyte (IFNα), fibroblast (IFNβ) and immune (IFNγ) interferon increase both the expression and shedding of the TAA BCA 225 by T47D cells and MCF-7 human breast carcinoma cells [36]. These compounds also increase the expression of the TAA carcinoembryonic antigen and HLA class II-DR antigen in both T47D and MCF-7 cells [22, 36]. The mechanism by which TPA induces its diversity of effects in target cells is believed to be mediated initially by its binding to the $Ca^{2+}$-

activated and phospholipid-dependent enzyme protein kinase C, (PKC), which is the high-affinity receptor for TPA (for review see [10, 43, 44]). As a consequence of activation of PKC many important biochemical processes are initiated in target cells, including both positive- and negative-feedback controls in signal transduction pathways (for review see [43, 44]). Recent studies have implicated PKC activation in mediating both antiviral activity and specific gene-regulatory changes induced by IFNα, IFNβ and IFNγ [6, 8, 37, 46, 47, 50] (and for review see [9]). The purpose of the present study was to explore the possible relationship between PKC activation and antigen up-regulation induced by phorbol esters and interferon. With this aim in mind we have determined the effect of the synthetic PKC activator 3-(*N*-acetylamino)-5-(*N*-decyl-*N*-methylamino)-benzyl alcohol (ADMB), the natural PKC activators TPA and mezerein and the combination of PKC activators and the PKC inhibitor H-7 on the antigenic phenotype of T47D cells. To determine if similar biochemical pathways are involved in the ability of IFNβ and IFNγ to alter the antigenic phenotype of T47D cells, we have also evaluated the effect of H-7 on interferon up-regulation of the same antigens in T47D cells.

## Materials and methods

*Cell cultures.* The T47D clone 11 human breast carcinoma cell line [32] was grown in RPMI-1640 medium supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, fungizone (0.25 μg/ml), streptomycin (50 μg/ml), penicillin (50 U/ml), 10% fetal bovine serum, β-estradiol and insulin (0.1 U/ml) at 37° C in a 5% $CO_2$/95% air humidified incubator. Cultures were maintained in the logarithmic phase of growth by subculturing at a 1 : 5 or 1 : 10 split ratio when cells approached confluence.

*Growth and [$^3$H]thymidine incorporation assays.* T47D cells were seeded at $5 \times 10^3$ cells/ml in 35-mm tissue-culture plates and 24 h later the medium was changed with the indicated compounds. After 72 h the cells were resuspended in trypsin/versene (0.125%/0.02%, w/w) and counted using a $Z_M$ Coulter Counter (Coulter Electronics). For [$^3$H]thymidine incorporation studies, T47D cells were seeded at $1.25 \times 10^4$ cells in 0.2 ml medium in 96-well microtiter plates. Every 24 h, plates received 1 μCi [*methyl*-$^3$H]thymidine (specific activity 10 μCi/mmol) (ICN Radiochemicals, Irvine, Calif), cells were harvested 8 h after the addition of labeled thymidine and trichloroacetic-acid-precipitable radioactivity [54] was determined using a Packard scintillation counter. Replicate samples varied by up to 10% and replicate studies varied by up to 15%.

*Monoclonal antibodies.* IgG1 murine mAb Cu18 and Cu46 recognize two different epitopes of a highly restricted breast-carcinoma-associated glycoprotein of $M_r$ 225000–250000 (BCA 225) [41]. This TAA is expressed intracytoplasmically and on the membrane of 94% of breast tumors tested and in the T47D human breast carcinoma cell line. BCA 225 is shed into the culture medium by T47D cells and into the sera of breast cancer patients. IgG2a mAb L243 [anti-(HLA class II-DR)] recognizes a monomorphic HLA class II-DRα epitope (ATCC M355). mAb CL203.4 [40], which recognizes intercellular adhesion molecule 1 (ICAM-1), was kindly provided by Dr. S. Ferrone, New York Medical College, N. Y. The c-*erb*B-2 mAb, which recognizes the extracellular domain of c-*erb*B-2, was obtained from Triton Biosciences Inc., Alameda, Calif. mAb Cu18 and Cu46 were used at 0.05 μg/ml and mAb interacting with HLA class II-DR, ICAM-1 and c-*erb*B-2 were used at 10 μg/ml. For each experiment, isotype-matched control backgrounds (IgG for Cu18, Cu46 and ICAM-1, IgG2a for HLA class II and IgG2b for c-*erb*B2) were subtracted from the experimental results. Background from anti-(mouse IgG) fluorescein-isothiocyanate(FITC)-labeled antibody was also subtracted from experimental results. We never observed backgrounds higher than 2% of total cells for isotypic mouse IgG or higher than 5% for anti-(mouse IgG)-FITC.

*Analysis of TAA and cellular antigen expression by fluorescence-activated cell sorter (FACS) analysis.* T47D cells treated with the various compounds were analyzed by flow cytometry using appropriate mAb concentrations as described previously [36]. Briefly, $1 \times 10^5$ cells were incubated with the test antibody for 30 min at 4° C, washed three times with phosphate-buffered saline (PBS) and incubated with a goat anti-[mouse F(ab)$_2$] FITC-conjugated test antibody at a 1 : 40 dilution for 30 min at 4° C. Cells were then washed three times with PBS analyzed on a FACStar (Becton Dickinson, Mountain View, Calif.) or a Coulter Epics IV FACS (Coulter, Hialeah, Fla.). Results are expressed as mean fluorescence intensity (MFI), which was determined as described previously [36]. MFI = (mean channel fluorescence in fluorescence-positive antibody-binding cells × fluorescence-positive antibody-binding cells, %) –(mean channel fluorescence in unstained cells × fluorescence-positive cells in the unstained population, %). All studies were performed a minimum of three or four times with duplicate samples in each experiment. Replicate samples within individual experiments varied by up to 10% and variation between experiments was generally no more than 20%.

*Analysis of the synthesis and shedding of BCA 225.* After appropriate incubation times with the various compounds, cell lysates were prepared from T47D cells. Cells were washed three times in PBS, pH 7.6, pelleted and incubated for 1 h at room temperature in 20 mM TRIS/HCl, pH 7.4, with phenylmethylsulfonyl fluoride PMSF. Cultures were then homogenized with a Teflon homogenizer, centrifuged at 3000 rpm for 10 min at 4° C and the supernatant was mixed 1 : 2 with 20 mM TRIS/HCl, pH 7.4, containing 0.5% NP40 (Sigma). After 1 h at 4° C, the mixture was spun at 3000 rpm for 10 min and the supernatant was passed through an Extractigel column (Pierce, Ill.) to remove excess detergent. Protein concentration was determined by the BCA micro-method (Pierce, Ill.). BCA 225 levels in cell extracts and supernatants from control and treated T47D cells were quantified using a double-determinant enzyme-linked immunosorbent assay (ELISA) [4, 36]. Briefly, Cu18-mAb-coated Nunc Immunoplates (Nunc, Denmark) were blocked with 1% bovine serum albumin (Sigma, radioimmunoassay reagent grade) and incubated with a 1 : 2 dilution of supernatant in duplicate. Standard values for a partially purified BCA 225 preparation were used at a range of 0–10 μg/ml in RPMI-1640 medium plus 10% fetal bovine serum (T47D growth medium). After a 2-h incubation and three washings with PBS/0.1% Tween 20, a Cu46 mAb conjugated to peroxidase was applied to the plate, incubated for 2 h, washed six times in PBS/0.1% Tween 20 and the reaction was developed with 16 ng orthophenyldiamino benzidine (OPD) (Sigma, St. Louis) and 4 μl 30% $H_2O_2$ in McIlvans buffer, pH 9.6. The plates were read on a Dynatech ELISA reader and a linear standard curve was generated and used to calculate the relative amount of BCA 225 in cell lysates and shed into the culture medium. Results were adjusted to nanograms of BCA 225 per milligram of protein, or per $10^6$ cells. Replicate samples varied by up to 10% and replicate experiments varied by up to 20%.

*RNA isolation and Northern blotting analysis.* Steady-state levels of HLA class I, HLA class II-DRβ, c-*erb*B-2 and ICAM-1 mRNA in control and treated T47D cells were determined by Northern blotting analysis of total cytoplasmic RNA probed with appropriate $^{32}$P-labeled gene probes as previously described [3, 56]. Northern blots were also probed with a $^{32}$P-labeled glyceraldehyde phosphate dehydrogenase [12, 55] gene probe to verify equal mRNA expression under various experimental conditions. Following hybridization, the filters were washed and exposed for autoradiography. Radioautograms were analyzed by densitometer to determine -fold change in mRNA expression as a result of treatment with the different antigenic modulating agents, with or without cocultivation with H-7.

*Reagents.* Recombinant human leukocyte (IFNαA) and immune (IFNγ) interferons were produced in *Escherichia coli* and purified to homogene-

**Table 1.** Effect of the protein kinase C (PKC) inhibitor H-7 on up-regulation of HLA class II antigens, c-*erb*B-2 and ICAM-1 by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and mezerein (MEZ) in T47D human breast carcinoma cells

| Experimental conditions[a] | Antigenic expression (MFI) | | |
|---|---|---|---|
| | HLA class II | c-*erb*B-2 | ICAM-1 |
| Control | 3410 | 7144 | 20975 |
| H-7 | 4536 (1.3)[b] | 7045 (1.0) | 21538 (1.0) |
| TPA | 9984 (2.9) | 16100 (2.3) | 63175 (3.0) |
| TPA+H-7 | 4778 (1.4) | 8385 (1.2) | 47542 (2.3) |
| MEZ | 10260 (3.0) | 18385 (2.6) | 85745 (4.1) |
| MEZ+H-7 | 3654 (1.1) | Not detected | 39486 (1.9) |

[a] T47D cells were grown for 72 h in 0.1 μg/ml TPA or 0.1 μg/ml MEZ, in the presence or absence of 0.1 μg/ml H-7. Cells were resuspended, incubated with mAb specific for HLA class II antigens, c-*erb*B-2 or ICAM-1 and fluorescein-isothiocyanate-labeled anti-(mouse Ig) secondary antibody. Cells were then analyzed by flow cytometry using a FACStar (Becton Dickinson, Mountain View, Calif.) and antigenic expression is expressed as mean fluorescence intensity (MFI). Further details can be found in Materials and methods.

[b] Numbers in brackets reflect the level of up-regulation versus untreated control cells (equivalent to 1.0)

ity as previously described [19, 49, 53]. These interferons were kindly provided by Dr. Sidney Pestka, UMDNJ–Robert Wood Johnson Medical School, Piscataway, N. J. Recombinant human fibroblast (IFNβ) interferon, with a serine substituted for a cysteine at position 17 of the molecule [39], was supplied by Triton Biosciences Inc., Alameda, Calif., as a lyophilized powder with a concentration of $4.5 \times 10^7$ units/ml. The interferon titers were determined by a cytopathic-effect-inhibition assay with vesicular stomatitis virus on a bovine kidney cell line (MDBK) or human fibroblast AG-1732 cells [49]. Concentrated stocks of interferons were divided into aliquots, frozen at –80° C, thawed immediately prior to use and diluted to the appropriate concentrations in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% or 10% fetal bovine serum. TPA, mezerein, and ADMB were obtained from LC Services Corp., Woburn, Mass. Stock solutions of 1 mg/ml (TPA and mezerein) or 10 mg/ml (ADMB) were prepared in dimethylsulfoxide, divided into aliquots and stored at –20° C. The PKC inhibitor H-7 [1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride] (Sigma) was prepared in distilled $H_2O$ and stored at 4° C. For experiments, aliquots were thawed and dispensed in DMEM containing 5% or 10% fetal bovine serum to yield appropriate final concentrations. The solvent dimethylsulfoxide (0.025%–0.05%) did not alter the growth or antigenic expression of T47D cells.

## Results

### *Effect of TPA, mezerein and ADMB on growth and DNA synthesis in T47D cells*

In preliminary studies, the dose range and time course of induction of antigenic enhancement in T47D cells by TPA, mezerein and ADMB, as well as the effect of various doses of H-7 on this process, was determined (data not shown). These studies indicated that the optimum effect on antigenic expression in T47D cells exposed to TPA, mezerein or ADMB occurred within 72 h. The most effective dose of TPA, mezerein and ADMB, inducing up-regulation of BCA 225, HLA class II antigens, ICAM-1 and c-*erb*B-2 in T47D cells, was found to be 0.1 μg/ml. The ability of these PKC activators to induce increased antigenic expression in

**Table 2.** Effect of the PKC inhibitor H-7 on the enhanced synthesis, expression and shedding of BCA 225 induced by TPA, mezerein and ADMB in T47D human breast carcinoma cells

| Experimental conditions[a] | BCA 225 synthesis[b] (ng/mg protein) | BCA 225 expression[c] (MFI) | BCA 225 shedding[d] (ng/ml/$10^6$ cells) |
|---|---|---|---|
| Control | 164 | 6426 | 28 |
| H-7 | 142 (0.9)[e] | 5428 (0.8) | 23 (0.8) |
| TPA | 394 (2.4) | 11696 (1.8) | 84 (3.0) |
| TPA+H-7 | 191 (1.2) | 7434 (1.2) | 35 (1.3) |
| MEZ | 507 (3.1) | 16065 (2.5) | 147 (5.3) |
| MEZ+H-7 | 225 (1.4) | 7068 (1.1) | 39 (1.4) |
| ADMB | 299 (1.8) | 12825 (2.0) | 26 (0.9) |
| ADMB+H-7 | 178 (1.1) | 7018 (1.1) | 34 (1.2) |

[a] T47D cells were incubated for 72 h in the presence of 0.1 μg/ml TPA, 0.1 μg/ml mezerein or 0.25 μg/ml ADMB, in the presence or absence of 0.1 μg/ml H-7

[b] Cell lysates were prepared and BCA 225 levels were determined by double-determinant BCA 225 ELISA assay as described in Materials and methods

[c] Membrane expression of BCA 225 was determined by flow cytometry using a FACStar (Becton Dickinson, Mountain View, Calif.) with CU18 mAb as described in Materials and methods. The results are expressed as mean fluorescence intensity (MFI)

[d] The shedding of BCA 225 into the culture medium was calculated using a double-determinant BCA 225 ELISA procedure as described in Materials and methods

[e] Values in brackets reflect the level of enhancement in BCA 225 relative to control (equivalent to 1.0)

T47D cells was inhibited by the simultaneous exposure to 0.1 μg/ml PKC inhibitor H-7 (Tables 1 and 2). The effect of TPA, mezerein, ADMB and H-7 on 72 h growth and [$^3$H]-thymidine incorporation in T47D cells is shown in Fig. 1. When exposed to 0.1 μg/ml of the respective compounds, growth and DNA synthesis were suppressed to the greatest degree in TPA-treated cells. In contrast, at the same dose of 0.1 μg/ml, mezerein and ADMB only marginally altered growth and DNA synthesis in T47D cells (Fig. 1). No additive or synergistic effect on 72-h growth suppression was observed when TPA or mezerein were used in combination with 0.1 μg/ml H-7 (data not shown).

### *Effect of TPA, mezerein and ADMB, alone and in combination with H-7, on the antigenic phenotype of T47D cells*

When tested for reactivity with specific monoclonal antibodies, control T47D cells displayed the following constitutive antigenic phenotype: 10%–20% of cells (with a mean channel fluorescence of 180–210) were positive for HLA class II antigen (HLA-DR) expression; 5%–10% (with a mean channel fluorescence of 110–130) were positive for c-*erb*B-2 expression; 80%–90% (with a mean channel fluorescence of 170–200) were positive for ICAM-1 expression; 85%–95% (with a mean channel fluorescence of 180–210) were positive for the TAA BCA 225 (as monitored by the mAb Cu18); and 60%–70% (with a mean channel fluorescence of 140–170) were positive for BCA 225 (as monitored by the mAb Cu46). The effect of TPA and mezerein, alone and in combination with

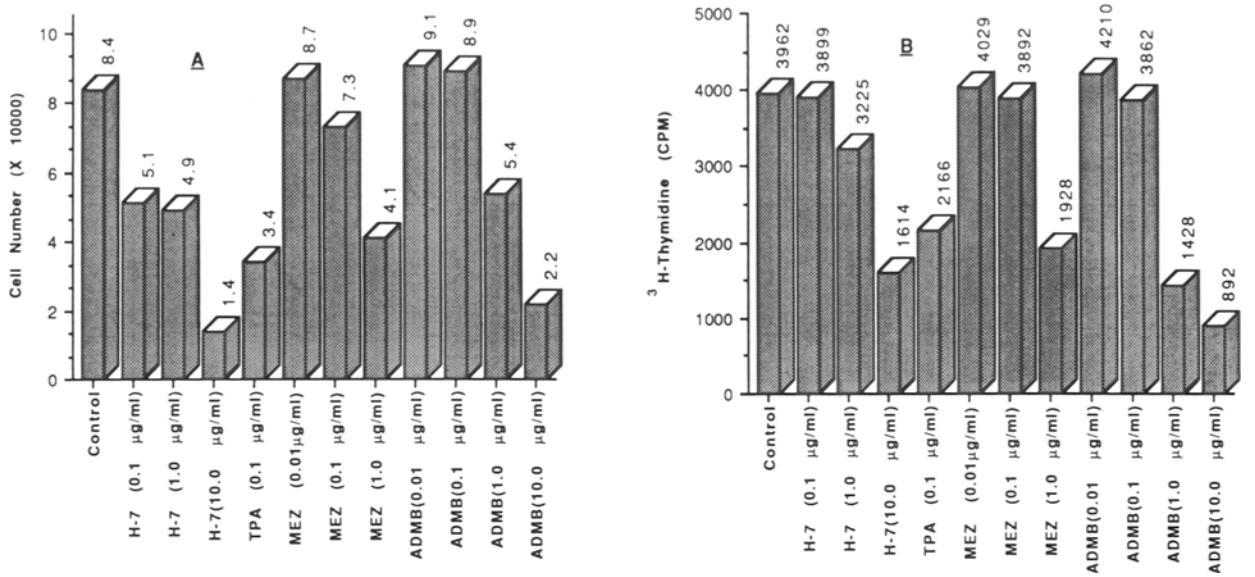


**Fig. 1 A, B.** Effect of 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (*H-7*), 12-*O*-tetradecanoylphorbol 13-acetate (*TPA*), mezerein (*MEZ*) and 3-(*N*-acetylamino)-5-(*N*-decyl-*N*-methylamino)-benzyl alcohol ADMB on T47D cell growth and DNA synthesis. Cell growth assays are presented in **A**. T47D cells were seeded at $1 \times 10^4$ cells/35-mm tissue-culture plate, the medium was changed with the indicated compounds 24 h later and cell numbers were determined by coulter counter after an additional 72 h growth at 37°C. Results are the average of triplicate samples per experimental condition, which varied by up to 10%. DNA synthesis assays are presented in **B**. T47D cells were seeded at $1.25 \times 10^4$ cells in 0.2 ml medium in 96-well microtiter plates. Every 24 h, cultures received 1 µCi [*methyl*-$^3$H]thymidine and 8 h later cells were harvested and trichloroacetic-acid-precipitable radioactivity was determined. Results are the average from replicate samples exposed to the indicated compounds for 72 h. Replicate samples varied by up to 10% and replicate studies varied by up to 15%. Further details can be found in Materials and methods

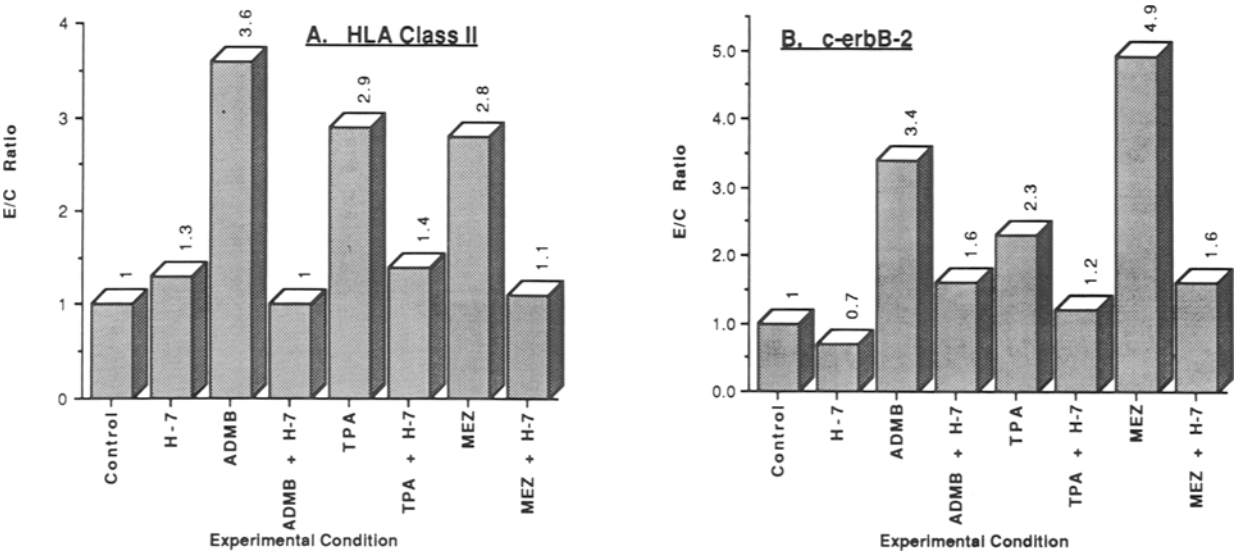


**Fig. 2.** Effect of H-7 on the up-regulation of HLA class II antigens and c-*erb*B-2 antigen expression in T47D cells induced by ADMB, TPA and mezerein. T47D cells were grown for 72 h in the presence of 0.1 µg/ml ADMB, TPA or mezerein, used alone or in combination with 0.1 µg/ml H-7. Cell-surface antigen expression was then determined by FACS analysis as described in Materials and methods. Base-line control antigen expression is given the value of 1.0. The values presented are the -fold change, which represents the ratio of the experimental mean fluorescence intensity (MFI) value versus the control MFI value for the specific antigen tested, in fluorescence in experimental versus control samples. The results presented are from a single experiment employing replicate samples. Similar results within 15% have been obtained in two additional studies. Further details can be found in Materials and methods

0.1 µg/ml H-7, on HLA class II antigens, c-*erb*B-2 and ICAM-1 expression in T47D cells is shown in Table 1. H-7 did not significantly alter the de novo expression of any of these antigens in T47D cells. However, when administered in conjunction with TPA or mezerein, H-7 effectively blocked the ability of these PKC stimulators to enhance antigenic expression. In the experiment shown in Table 1, mezerein was somewhat more effective than TPA in enhancing c-*erb*B-2 and ICAM-1 expression. An increased activity, at comparable doses, of mezerein over TPA in enhancing the expression of these antigens, as well as the TAA BCA 225, has been found in several additional stud-

ies (unpublished data, Table 2 and Fig. 2). For comparison purposes, a single experiment is shown in Table 1. In this experiment, the combination of mezerein + H-7 resulted in the lack of detectable c-*erb*B-2 expression. This result may reflect technical difficulties rather than a complete suppression in c-*erb*B-2 expression, since in additional studies H-7 blocked the ability of mezerein to enhance the expression of this antigen without completely eliminating c-*erb*B-2 expression (unpublished data and Fig. 2).

Recent computer modeling studies have resulted in the synthesis of compounds that inhibit the binding of phorbol esters to PKC and activate PKC in platelets resulting in the phosphorylation of a specific 40-kDa protein substrate [58]. We have presently tested one of these phorbol ester pharmacophores, ADMB, for its ability to up-regulate the same antigens in T47D cells previously shown to be modulated by TPA and mezerein. A comparison of the efficacy of up-regulation of HLA class II antigens and c-*erb*B-2 in T47D cells exposed to TPA, mezerein and ADMB, in the presence or absence of H-7, is presented in Fig. 2. In the case of HLA class II antigens, ADMB was somewhat more effective than TPA and mezerein in inducing up-regulation, whereas H-7 reduced or eliminated enhancement when applied in combination with these PKC activators. In the case of c-*erb*B-2, mezerein was the most effective PKC activator tested in enhancing expression and, as observed with HLA class II antigens, H-7 reduced this antigenic upregulation.

A series of experiments were conducted to determine the effect of TPA, mezerein and ADMB, alone and in combination with H-7, on the synthesis, surface expression and shedding of the TAA BCA 225 (Table 2). The synthesis of BCA 225 was increased following exposure to all of the PKC activators, with mezerein being most effective in enhancing the synthesis of this TAA. Similarly, mezerein was the most effective of the three PKC activators in enhancing the surface expression of BCA 225 in T47D cells. As observed with the other antigens analyzed, H-7 effectively blocked both the enhanced synthesis and surface expression of BCA 225. When compared for their ability to induce shedding of BCA 225 from T47D cells, a differential response was observed between the three PKC activators (Table 2). Both mezerein and TPA enhanced shedding of BCA 225, with mezerein again being more effective than TPA, whereas ADMB did not induce this effect in T47D cells. As observed with both synthesis and shedding, H-7 reduced the ability of mezerein and TPA to induce shedding of BCA 225.

*Effect of IFNβ and IFNγ, alone and in combination with H-7, on the antigenic phenotype of T47D cells*

We have previously demonstrated that both IFNβ and IFNγ can effectively enhance the expression of BCA 225, HLA class II antigens and ICAM-1 expression in T47D cells [36]. Optimum enhancement was observed by 72 h with ranges of interferon of 500–1000 units/ml IFNα or IFNβ and 50–500 units/ml IFNγ. The effect of 500 units/ml IFNβ and 50 units/ml IFNγ, in the presence or absence of 1.0 μg/ml H-7, is shown in Tables 3 and 4. A higher dose of H-7 was employed in this study because the lower H-7 concentration of 0.1 μg/ml did not block enhanced antigenic expression in interferon-treated T47D cells (data not shown). IFNγ was more effective (even at lower concentrations) than IFNβ in enhancing the expression of BCA 225, HLA class II antigens and ICAM-1. IFNα also increased the expression of the three antigens tested, but to a lower extent than IFNβ or IFNγ (data not shown). For example, in the experiment shown in Table 3, 500 units/ml IFNα enhanced HLA class II expression from an MFI of 2814 to an MFI of 11660 (a 4-fold increase) as opposed to a 11- and a 69-fold increase, respectively, in HLA class II expression in cells exposed to IFNβ or IFNγ. In a number of experiments, the de novo level of expression of specific

**Table 3.** Effect of the PKC Inhibitor H-7 on up-regulation of HLA class II antigens and ICAM-1 by IFNβ and IFNγ in T47D human breast carcinoma cells

| Experimental conditions[a] | Antigenic expression (MFI) | |
|---|---|---|
| | HLA class II | ICAM-1 |
| Control | 2919 | 54536 |
| H-7 | 3490 (1.2)[b] | 54080 (1.0) |
| IFNβ | 31413 (10.8) | 97013 (1.8) |
| IFNβ+H-7 | 32190 (11.1) | 108902 (2.0) |
| IFNγ | 202102 (69.2) | 191490 (3.5) |
| IFNγ+H-7 | 210812 (75.2) | 188544 (3.5) |

[a] T47D cells were incubated for 72 h in the presence of 500 units/ml IFNβ or 50 units/ml IFNγ, in the presence or absence of 1.0 μg/ml H-7. Cells were then incubated with mAb specific for HLA class II antigens or ICAM-1 followed by fluorescinated anti-(mouse IgG) antibody and then analyzed by flow cytometry with a Coulter Epics IV FACS (Coulter Electronics, Hialeah, Fla.) as described in Materials and methods. Results are expressed as mean fluorescence intensity (MFI)

[b] Values in brackets indicate the relative increase in expression versus untreated controls (equivalent to 1.0)

**Table 4.** Effect of the PKC inhibitor H-7 on the enhanced synthesis, expression and shedding of BCA 225 induced by IFNβ and IFNγ in T47D human breast carcinoma cells

| Experimental conditions[a] | BCA 225 synthesis[b] (ng/mg protein) | BCA 225 expression[c] (MFI) | BCA 225 shedding[d] (ng/ml/$10^6$ cells) |
|---|---|---|---|
| Control | 223 | 2816 | 47 |
| H-7 | 230 (1.0)[e] | 2560 (0.9) | 38 (0.8) |
| IFNβ | 374 (1.7) | 5716 (2.0) | 69 (1.5) |
| IFNβ+H-7 | 361 (1.6) | 5714 (2.0) | 66 (1.4) |
| IFNγ | 722 (3.2) | 9069 (3.2) | 64 (1.4) |
| IFNγ+H-7 | 694 (3.1) | 8177 (2.9) | 64 (1.4) |

[a] T47D cells were incubated for 72 h with 500 units/ml IFNβ or 50 units/ml IFNγ, in the presence or absence of 1.0 μg/ml H-7

[b] BCA 225 synthesis was calculated by ELISA using cell lysates as described in Materials and methods

[c] BCA 225 cells-surface expression was measured by flow cytometry using a FACStar (Becton Dickinson, Mountain View, Calif.) as described in Materials and methods. Results are expressed as mean flurorescence intensity (MFI)

[d] Shedding of BCA 225 into the culture medium was measured by ELISA as described in Materials and methods

[e] Values in brackets indicate the relative change in BCA 225 in comparison with controls (equivalent to 1.0)

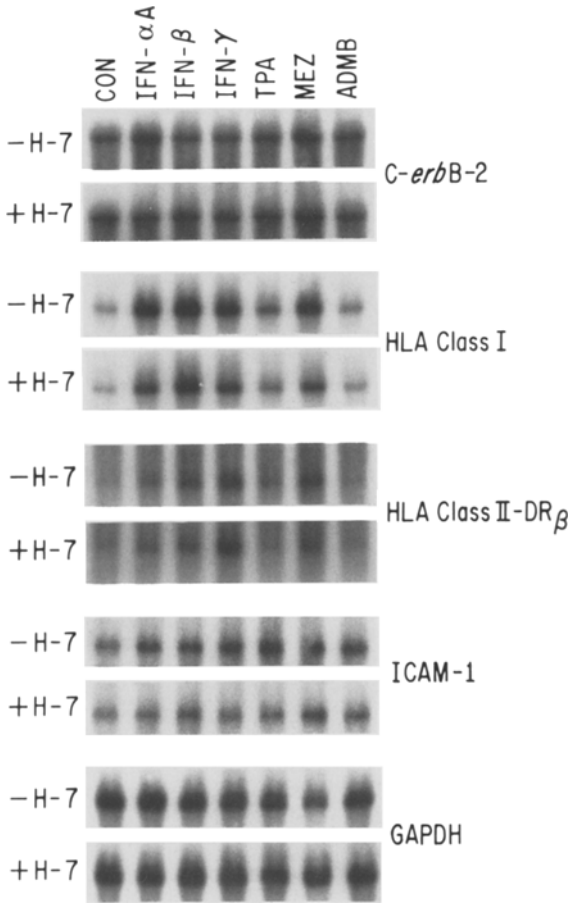


**Fig. 3.** Effect of interferon, ADMB, TPA and mezerein, alone and in combination with H-7, on steady-state mRNA levels of HLA class I antigens, HLA class II-DRβ antigen, c-*erb*B-2, intercellular adhesion molecule 1 (*ICAM-1*) and glyceraldehyde phosphate dehydrogenase (*GAPDH*) in T47D human breast carcinoma cells. T47D cells were grown for 72 h in the absence (control) or presence of 500 units/ml IFNα or IFNβ, 50 units/ml IFNγ, 0.1 μg/ml TPA, mezerein or ADMB. Cultures were also grown in the presence of 0.1 μg/ml H-7 for 72 h with and without the additional compounds indicated above. Total cytoplasmic RNA was isolated and processed as described in Materials and methods. Control, *CON*; recombinant human leukocyte interferonA, *IFN-αA*; recombinant human fibroblast interferon, *IFNβ*; recombinant human immune interferon, *IFN-γ*; *ADMB*, 3-(*N*-acetylamino)-5-(*N*-decyl-*N*-methylamino)-benzyl alcohol; *TPA*, 12-*O*-tetradecanoylphorbol 13-acetate; *MEZ*, mezerein; H-7, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride

antigens and the absolute level of up-regulation varied. Part of this difference may reflect the use of a different FACS with different sensitivities for determining MFI and/or innate differences in antigenic expression of cells as a consequence of the cell cycle. Although this makes it difficult to compare directly absolute levels of up-regulation with the phorbol esters and ADMB versus the interferons, it still permits a comparison of the effect of H-7 on up-regulation. In experiments simultaneously comparing the various compounds, IFNγ was generally a more effective enhancer of HLA class II antigens and ICAM-1 than the other agents, whereas mezerein was generally more effective in modifying c-*erb*B-2 and BCA 225 expression. Unlike antigenic up-regulation induced by the phorbol esters and ADMB, which was inhibited by H-7, even the higher dose of H-7 (1.0 μg/ml) did not inhibit the ability of IFNβ or IFNγ to enhance BCA 225, HLA class II antigens and ICAM-1 expression in T47D cells.

To determine if H-7 could inhibit the ability of interferon to enhance the synthesis or shedding of BCA 225 in T47D cells, cultures were grown for 72 h in the presence of 500 units/ml IFNβ or 50 units/ml IFNγ and in the presence or absence of 1 μg/ml H-7 (Table 4). As had been observed for interferon-enhanced expression of BCA 225, H-7 did not inhibit the synthesis or shedding of BCA 225 induced in T47D cells by interferon. These results demonstrate that the ability of TPA and mezerein versus IFNβ and IFNγ to up-regulate the synthesis, expression and shedding of BCA 225 may arise from different mechanisms.

*Effect of ADMB, TPA, mezerein and interferon, alone and in combination with H-7, on the steady-state levels of HLA class I antigens, HLA class II-DRβ antigen, ICAM-1 and c-erbB-2 mRNA in T47D cells*

To determine if the increase in HLA class II-DRβ antigen, ICAM-1 and c-*erb*B-2 expression in T47D cells resulting from 72 h ADMB, TPA, mezerein, IFNα, IFNβ or IFNγ treatment involved enhanced mRNA expression, the steady-state levels of mRNA for the respective genes were determined (Fig. 3). In the case of HLA class II-DR antigen, small increases in mRNA levels were observed in T47D cells treated with IFNα (1.1-fold), IFNβ (1.2-fold), IFNγ (1.25-fold) and mezerein (1.2-fold). ADMB and TPA did not increase HLA class II-DRβ antigen mRNA expression, although ADMB was slightly more effective than mezerein in enhancing cell-surface expression of this antigen in T47D cells (Fig. 2). Mezerein and IFNγ were the most effective enhancers of HLA class II-DRβ antigen mRNA expression, whereas IFNγ was more effective than mezerein in enhancing expression of this antigen in T47D cells (Tables 1 and 3). When cotreated with the respective antigenic modulating compound and H-7, only minimal changes in HLA class II-DRβ antigen mRNA levels (<1.15-fold) were observed. These observations are in contrast to the HLA class II-DR antigenic modulation induced by these agents in T47D cells. As described above, H-7 effectively blocked ADMB, TPA and mezerein enhancement of HLA class II-DR antigen expression (Table 1 and Fig. 2), whereas H-7 did not inhibit the ability of IFNβ or IFNγ to augment class II HLA-DR antigen expression in T47D cells (Table 3). In the case of HLA class I antigen expression, mRNA levels were variably increased following treatment with IFNα (1.9-fold), IFNβ (2.1-fold), IFNγ (1.8-fold), TPA (1.3-fold) and mezerein (1.75-fold), whereas H-7 only marginally altered mRNA levels ($\leqslant$1.2-fold) for this antigen (Fig. 3). With respect to surface expression, as observed with HLA class II-DR antigen expression, H-7 inhibited up-regulation induced by ADMB, TPA or mezerein, but not up-regulation induced by the interferons (data not shown). These observations indicate that cell-surface expression changes in both HLA class I antigens and HLA class II-DR antigen, induced by the various compounds in T47D cells, are not directly related

to large changes in mRNA levels for these antigens. Similarly, the ability of H-7 to block ADMB-, TPA- and mezerein-enhanced expression of both HLA class I antigens and HLA class II-DR antigen in T47D cells is not a consequence of a reduction in mRNA levels for these gene products. Further studies are required, however, to determine if the antigenic modulating agents, employed alone or in combination with H-7, modulate the rate of mRNA synthesis and/or decay of mRNA synthesis for HLA class II-DR or HLA class I antigens in T47D cells.

ICAM-1 mRNA levels were increased a maximum of only 1.3-fold after 72 h treatment under the various experimental conditions and H-7 only modestly altered ICAM-1 expression (Fig. 3). In the case of c-*erb*B2, a maximum increase of only 1.2-fold in the levels of mRNA was apparent after 72 h treatment with the various agents. Similarly, no differential change in c-*erb*B-2 mRNA was observed in T47D cells grown in the presence of any of the antigenic modulating agents plus H-7. These results again contrast with those measuring surface expression of these antigens. As demonstrated in Tables 1 and 3, H-7 partially inhibited enhanced ICAM-1 surface expression in T47D cells treated simultaneously with ADMB or mezerein, whereas it was ineffective in inhibiting IFNβ- or IFNγ-enhanced ICAM-1 expression (Table 3). Similarly, H-7 inhibited enhanced surface expression of c-*erb*B2 induced by ADMB, TPA and mezerein (Fig. 2 and Table 1), whereas it did not alter up-regulation induced by the interferons (data not shown). These results provide further support for the lack of a direct correlation between the levels of ICAM-1 and c-*erb*B-2 mRNA and antigenic expression in cells treated with the combination of ADMB, TPA, mezerein or interferon and H-7. Further studies are required, however, to determine if the antigenic modulating agents, employed alone or in combination with H-7, modulate the rate of mRNA synthesis and/or decay of mRNA synthesis for ICAM-1 or c-*erb*B2 in T47D cells.

## Discussion

Among the diversity of interferon effects on target cells, recent investigations have focused on the ability of these bioresponse modulators to enhance the expression of both histocompatibility antigens and TAA in tumor cells (for review see [1, 9, 21, 25, 28]). These studies indicate that interferon may prove valuable in altering the phenotype of tumor cells rendering them more accessible to mAb targeting [13, 23, 36, 42]. A frequent observation is that interferon functions predominantly as an enhancer of antigenic expression, rather than an inducer of de novo expression of specific antigens [9, 15, 16, 25, 31]. At the present time, little information is available on the biochemical mechanisms underlying interferon up-regulation of antigenic expression. Studies comparing the protein synthesis requirements for antigenic modulation induced by types I (IFNα/β) and type II (IFNγ) interferon in human melanoma cells suggest that different biochemical pathways mediate up-regulation of both major-histocompatibility-complex(MHC)- and non-MHC-encoded glycoproteins induced by these compounds [16]. IFNγ enhancement of antigen expression depends on continued protein synthesis, whereas the modulatory effect of IFNα and IFNβ can occur in the presence of the protein synthesis inhibitor cycloheximide. Numerous studies have also indicated that types I and II interferons can differ in their effects on TAA expression in the same tumor cell (for review see [1, 21, 25, 48]). Both the absolute level of antigenic modulation induced by different interferons, as well as the type of effect elicited in specific target cells, i.e. either stimulatory or inhibitory, has been shown to vary (for review see [1, 21]). In the present study we have addressed the potential relationship between PKC activation and antigenic modulation induced by recombinant IFNβ and IFNγ in the human breast carcinoma cell line T47D. Since both TPA and mezerein can augment the expression of the same histocompatibility antigens and TAA in T47D cells as recombinant interferons, and these agents appear to work directly via activation of PKC (for review see [43, 44], we have also incorporated these agents in our studies.

The enhanced cellular antigenic expression induced in T47D cells by TPA, mezerein and ADMB was eliminated by simultaneous incubation with the PKC inhibitor H-7. Similarly, the ability of TPA and mezerein to enhance the synthesis and shedding of the TAA BCA 225 was also blocked by H-7. In contrast, ADMB failed to induce increased shedding of BCA 225 by T47D cells, whereas it was as active as TPA in enhancing BCA 225 synthesis and expression (Table 2). These observations suggest that ADMB can differentially modify the antigenic phenotype in T47D cells in comparison with TPA and mezerein, i.e. it can augment synthesis and expression without enhancing shedding of specific TAA. Interestingly, ADMB was found to induce HLA class II antigens in a similar manner to TPA and mezerein in T47D cells. HLA class II antigens have been shown to be involved in the differentiation of mammary epithelium [11] and they play a critical role in antigen presentation to T cells [35], the transport of key intracellular peptides to the extracellular milieu [57] and recruitment of lymphoid cells to tumor cells [11, 33, 59]. The ability of ADMB to enhance both HLA class II antigens and TAA expression on T47D cells could have implications with respect to the induction of an immune response to this tumor in vivo. A severe limitation preventing the use of TPA or mezerein as potential immunomodulators in humans is their well-documented tumor-promoting activity in the mouse skin two-stage carcinogenesis assay (for review see [51, 52]). At the present time, ADMB has not been tested for in vivo toxicity, tumor-promoting activity and/or in vivo immunomodulatory properties. However, if this compound can pass this scrutiny, it could prove useful as an antigenic modulating agent in situations where increased surface expression without a concomitant increase in TAA shedding is desired.

Previous studies have indicated a possible involvement of activation of PKC in the early events associated with the action of IFNα in specific target cells [2, 6, 7, 46, 50, 60–62]. Although not studied as extensively, an association between PKC activity and both IFNβ- and IFNγ-induced cellular changes has also been suggested [8, 29, 30, 45, 47]. In addition, a differential role for PKC in IFNα/β- versus IFNγ-induced cellular and gene expression changes

in the same target cell has also been observed [6, 37, 47]. Both IFNβ and IFNγ produced similar antigenic changes in T47D cells to those brought about by TPA and mezerein, including enhancing the shedding of BCA 225. However, up-regulation of antigen expression and increased shedding induced by IFNβ and IFNγ were not inhibited by H-7 (Table 4). IFNα was less effective than either IFNβ or IFNγ in modifying the antigenic phenotype of T47D cells and its activity also was not blocked by H-7 (data not shown). These results suggest that the mechanism by which interferons modulate antigen expression in T47D cells occurs by a PKC-independent pathway. A similar dissociation between PKC activation and the ability of IFNγ and TPA to induce specific antigenic expression changes in human keratinocyte cultures has recently been reported [24]. IFNγ and TPA both enhanced ICAM-1 expression in human keratinocyte cultures and the enhancement effect of TPA, but not that of IFNγ, was inhibited by H-7. Similarly, only IFNγ induced HLA class II-DR antigen expression in human keratinocytes and H-7 also failed to block this induction. Koide et al. [34] demonstrated that IFNγ induction of HLA class II-DR antigen expression in HL-60 cells also was not modified by H-7. In contrast, W-7 (a calmodulin antagonist) blocked IFNγ induction of HLA class II-DR expression in HL-60 cells supporting a possible involvement of calcium/calmodulin in antigenic modulation in this cell line. Similarly, treatment of murine macrophages with IFNγ resulted in the induction of both increased mRNA and MHC I-Aβ antigen expression, and both of these parameters were unaltered in the presence of H-7 [5]. W-7 did, however, modify the MHC I-Aβ antigen mRNA induction process elicited by IFNγ treatment in murine macrophages. In the case of the human melanoma cell line H0-1, the enhanced expression of HLA class I antigens, HLA class II antigens and ICAM-1 induced by IFNγ was again only marginally affected by H-7 [28]. Since up-regulation of antigen expression in T47D cells induced by PKC activators such as TPA, mezerein and ADMB are inhibited by H-7, whereas similar changes induced by the interferons are not blocked by H-7, these results further indicate that the mechanism controlling antigenic modulation in specific cell cultures is dependent on the specific inducer employed and antigenic modulation can occur by both a PKC-independent and a PKC-dependent pathway.

The mechanism by which ADMB and mezerein versus IFNγ enhance the expression of specific cellular antigens and TAA in T47D is not presently known. Analysis of steady-state mRNA levels of HLA class I, HLA class II-DRβ, ICAM-1 and c-*erb*B-2 in cells treated with these different compounds indicated various levels of modulation that did not correlate directly with the relative level of change in surface expression of these antigens. Similarly, H-7 did not significantly alter the level of mRNA for the various antigens under any of the experimental conditions. These results suggest that the ability of H-7 to modulate the antigenic enhancing properties of ADMB, TPA, mezerein and the interferons may occur at a post-transcriptional level. Alternatively, these agents may modify antigenic expression by altering the rate of mRNA transcription and/or mRNA stability. In the case of BCA 225, H-7 may exert its suppressive effect on ADMB-, TPA- and mezerein-induced increases in surface expression by inhibiting the ability of these compounds to enhance the synthesis of this TAA in T47D cells. Alternatively, H-7 might block antigenic up-regulation in ADMB-, TPA- and mezerein-treated cells by preventing the necessary biochemical alterations responsible for the insertion of the various antigens into the cell membrane in a form recognized by the mAb employed. Further studies are clearly required to determine the mechanism by which specific antigenic modulators up-regulate antigen expression and the mechanism by which H-7 selectively inhibits this process in cells treated with ADMB, TPA or mezerein. The present model system should prove useful in determining the biochemical mechanism(s) underlying antigenic up-regulation in response to diverse transmembrane signalling agents. With this information it may be possible in the future to design strategies and molecules specifically tailored to alter the antigenic phenotype of tumor cells making them more accessible to mAb-targeted therapeutic approaches.

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## References

1. Ahmed MA, Nielsch U, Guarini L, Hermo H Jr, Fisher PB (1990) Modulation of differentiation: a potential mechanism by which interferons induce antitumor activity. In: Fisher PB (ed) Mechanisms of differentiation: II. Modulation of differentiation by exogenous agents. CRC, Boca Raton, pp 1–56
2. Akai H, Larner A (1989) Phorbol ester-mediated down-regulation of an interferon-inducible gene. J Biol Chem 264: 3252
3. Babiss LE, Young CSH, Fisher PB, Ginsberg HS (1983) Expression of adenovirus E1A and E1B gene products and the *Escherichia coli* XGPRT gene in KB cells. J Virol 46: 454
4. Blazar BA, Sutton LM, Strome M (1983) Self-stimulating growth factor production by B-cell lines derived from Burkitt's lymphomas and other cell lines transformed in vitro by Epstein-Barr virus. Cancer Res 43: 4562
5. Celada A, Maki RA (1991) IFN-γ induces the expression of the genes for MHC Class II I-Aβ and tumor necrosis factor through a protein kinase C-independent pathway. J Immunol 146: 114
6. Csermely P, Balint E, Grimley PM, Aszalos A (1990) Protein kinase C is involved in the early signals of interferon-α but not interferon-γ in U937 cells. J Interferon Res 10: 605
7. Faltynek CR, Princler GL, Gusella GL, Varesio L, Radzioch D (1989) A functional protein kinase C is required for induction of 2-5A synthetase by recombinant interferon-αA in Daudi cells. J Biol Chem 264: 14 305
8. Fan X-D, Goldberg M, Bloom BR (1988) Interferon-γ-induced transcriptional activation is mediated by protein kinase C. Proc Natl Acad Sci USA 85: 5122
9. Fisher PB, Rowley PT (1991) Regulation of growth, differentiation and antigen expression in human tumor cells by recombinant cytokines: applications for the differentiation therapy of human cancer. In: Waxman S, Rossi GB, Takaku F (eds) Status of differentiation therapy, vol II. Raven, New York, pp 201–213
10. Fisher PB, Schachter D, Mufson RA, Huberman E (1989) The role of membrane lipid dynamics and translocation of protein kinase C in the induction of differentiation in human promyelocytic leukemic cells.

In: Kabara JJ (ed) Pharmacological effect of lipids: III. role of lipids in cancer research, American Oil Chemists' Society, Champaign, Ill., pp 69–89
11. Forsum U, Claesson K, Mjelm E, Karlsson-Parra A, Klareskog L, Scheynius A, Tjernlund U (1985) Class II transplantation antigens: distribution in tissues and involvement in disease. Scand J Immunol 21: 389
12. Fort P, Marty L, Piechaczyk M, Sabrouty SE, Dani C, Jeanteur P, Blanchard JM (1985) Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. Nucleic Acids Res 13: 1431
13. Fuith LC, Marth C, Muller-Holzner E, Zechmann W, Daxenbichler G (1991) Enhancement of CA 125 expression by interferon-gamma in ovarian carcinoma xenografts. J. Tumor Marker Oncol 6: 85
14. Giacomini P, Aguzzi A, Pestka S, Fisher PB, Ferrone S (1984) Modulation by recombinant DNA leukocyte (α) and fibroblast (β) interferons of the expression and shedding of HLA and tumor associated antigens by human melanoma cells. J Immunol 133: 1649
15. Giacomini P, Fisher PB, Duigou GJ, Gambari R, Natali PG (1988) Regulation of class II MHC gene expression by interferons: insights into the mechanism of action of interferon. Anticancer Res 8: 1153
16. Giacomini P, Tecce R, Sacchi A, Fisher PB, Natali PG (1988) Recombinant human IFN-γ, but not IFN-α or IFN-β, enhances MHC and non MHC encoded glycoproteins by a protein synthesis dependent mechanism. J Immunol 140: 3073
17. Giacomini P, Fraioli R, Nistico P, Tecce R, Nicotra MR, Di Filippo F, Fisher PB, Natali PG (1990) Modulation of the antigenic phenotype of early-passage human melanoma cells derived from multiple autologous metastases by recombinant human leukocyte, fibroblast and immune interferon. Int J Cancer 46: 539
18. Graham GM, Guarini L, Moulton TA, Datta S, Ferrone S, Giacomini P, Kerbel RS, Fisher PB (1991) Potentiation of growth suppression and modulation of the antigenic phenotype in human melanoma cells by the combination of recombinant human fibroblast and immune interferons. Cancer Immunol Immunother 32: 382
19. Gray PW, Leung B, Pennica D, Yelverton E, Najarian R, Simonsen CC, Derynk R, Sherwood PJ, Wallace DM, Berger SL, Levinson AD, Goeddel DV (1982) Expression of human immune interferon cDNA in *E. coli* and monkey cells. Nature 295: 502
20. Greiner JW, Hand PH, Noguchi P, Fisher PB, Pestka S, Schlom J (1984) Enhanced expression of surface tumor-associated antigens on human breast and colon tumor cells after recombinant leukocyte α-interferon treatment. Cancer Res 44: 3208
21. Greiner JW, Schlom J, Pestka S, Langer JA, Giacomini P, Kusama M, Ferrone S, Fisher PB (1985) Modulation of tumor associated antigen expression and shedding by recombinant human leukocyte and fibroblast interferons. Pharmacol Ther 31: 209
22. Greiner JW, Fisher PB, Pestka S, Schlom J (1986) Differential effects of recombinant human leukocyte interferons on cell surface antigen expression. Cancer Res 46: 4894
23. Greiner JW, Guadagni F, Noguchi P, Pestka S, Colcher D, Fisher PB, Schlom J (1987) Use of recombinant interferon to enhance monoclonal antibody-targeting of carcinoma lesions in vivo. Science 235: 895
24. Griffiths CEM, Esmann J, Fisher GJ, Voorhees JJ, Nickoloff BJ (1990) Differential modulation of keratinocyte intercellular adhesion molecule-1 expression by gamma interferon and phorbol ester: evidence for involvement of protein kinase C signal transduction. Br J Dermatol 122: 333
25. Guadagni F, Kantor J, Schlom J, Greiner JW (1990) Regulation of tumor antigen expression by recombinant interferons. In: Fisher PB (ed) Mechanisms of differentiation: II. Modulation of differentiation by exogenous agents. CRC, Boca Raton, pp 57–80
26. Guarini L, Temponi M, Edwalds GM, Vita JR, Fisher PB, Ferrone S (1989) In vitro differentiation and antigenic changes in human melanoma cell lines. Cancer Immunol Immunother 30: 262
27. Guarini L, Temponi M, Bruce JN, Bollon AP, Duigou GJ, Moulton TA, Ferrone S, Fisher PB (1990) Expression and modulation by cytokines of the intercellular adhesion molecule-1 (ICAM-1) in human central nervous system tumor cell cultures. Int J Cancer 46: 1041
28. Guarini L, Graham GM, Jiang H, Ferrone S, Zucker S, Fisher PB (1992) Modulation of the antigenic phenotype of human melanoma cells by differentiation-inducing and growth-suppressing agents. Pigment Cell Res Suppl 2: 123
29. Hamilton TA, Becton DL, Somers SD, Gray PW, Adams DO (1985) Interferon-γ modulates protein kinase C activity in murine peritoneal macrophages. J Biol Chem 260: 1378
30. Ito M, Takami Y, Tanabe F, Shigeta S, Tsukui K, Kawade Y (1988) Modulation of protein kinase C activity during inhibition of tumor growth by IFN-β and IFN-γ. Biochem Biophys Res Commun 150: 126
31. Kantor J, Tran R, Greiner J, Pestka S, Fisher PB, Schlom J (1989) Modulation of carcinoembryonic antigen messenger RNA levels in human colon carcinoma cells by recombinant human γ-interferon. Cancer Res 49: 2651
32. Keydar I, Ohno T, Nayak T, Sweet R, Simoni F, Weiss F, Karby S, Mesa-Tejada R, Spiegelman S (1984) Properties of retrovirus-like particles produced by a human breast carcinoma cell line: immunologic relationship with mouse mammary tumor virus proteins. Proc Natl Acad Sci USA 81: 4188
33. Klareskog L, Forsum U, Peterson PA (1985) Hormonal regulation of the expression of Ia antigens on mammary gland epithelium. Eur J Immunol 10: 958
34. Koide Y, Ina Y, Nezu N, Yoshida TO (1988) Calcium influx and $Ca^{2+}$-calmodulin complex are involved in interferon-γ-induced expression of HLA Class II molecules on HL-60 cells. Proc Natl Acad Sci USA 85: 3120
35. Landei M, Lamb JR, Bottazo GF, Feldman M (1984) Epithelial cells expressing aberrant MHC Class II determinants can present antigen to cloned human T-cells. Nature 312: 639
36. Leon JA, Mesa-Tejada R, Gutierrez MC, Estabrook A, Greiner JW, Schlom J, Fisher PB (1989) Increased surface expression and shedding of tumor associated antigens by human breast carcinoma cells treated with recombinant human interferons or phorbol ester tumor promoters. Anticancer Res 9: 1639
37. Lew DJ, Decker T, Darnell JE Jr (1989) Alpha interferon and gamma interferon stimulate transcription of a single gene through different signal transduction pathways. Mol Cell Biol 9: 5404
38. Maio M, Gulwani B, Langer JA, Kerbel RS, Duigou GJ, Fisher PB, Ferrone S (1989) Modulation by interferons of HLA antigens, high molecular weight-melanoma associated antigens and intercellular adhesion molecule-1 expression by cultured melanoma cells with different metastatic potential. Cancer Res 49: 2980
39. Mark DV, Lu SD, Creasey A, Yamamoto R, Lin L (1984) Site-specific mutagenesis of the human fibroblast interferon gene. Proc Natl Acad Sci USA 81: 5662
40. Matsui M, Temponi M, Ferrone S (1987) Characterization of a monoclonal antibody-defined human melanoma-associated antigen susceptible to modulation by immune interferon. J Immunol 139: 2088
41. Mesa-Tejada R, Palakodety RB, Leon JA, Khatcherian AO, Greaton CJ (1988) Immunocytochemical distribution of a breast carcinoma associated glycoprotein identified by monoclonal antibodies. Am J Pathol 130: 305
42. Murray JL, Zukiwski AA, Mujoo K, Rosenblum MG (1990) Recombinant α-interferon enhances tumor targeting of an antimelanoma monoclonal antibody in vivo. J Biol Response Mod 9: 556
43. Nishizuka YJ (1986) Studies and perspectives of protein kinase C. Science 233: 305
44. Nishizuka YJ (1988) The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature 334: 661
45. Ostrowski J, Meier KE, Stanton TH, Smith LL, Bomsztyk K (1988) Interferon-γ and interleukin-1α induce transient translocation of protein kinase C activity to membranes in a B lymphoid cell line. J Biol Chem 263: 13 786
46. Pfeffer LM, Strulovici B, Saltiel AR (1990) Interferon-α selectively activates the β isoform of protein kinase C through phosphatidylcholine hydrolysis. Proc Natl Acad Sci USA 87: 6537

47. Razdioch D, Varesio L (1988) Protein kinase C inhibitors block the activation of macrophages by IFN-β but not by IFN-γ. J Immunol 140: 1259
48. Reddy PG, Graham GM, Datta S, Guarini L, Moulton TA, Jiang H, Gottesman MM, Ferrone S, Fisher PB (1991) Effect of recombinant fibroblast interferon and recombinant immune interferon on growth and the antigenic phenotype of multidrug-resistant human glioblastoma multiforme cells. J Natl Cancer Inst 83: 1307
49. Rehberg E, Kelder B, Hoal EG, Pestka S (1982) Specific molecular activities of recombinant and hybrid leukocyte interferons. J Biol Chem 257: 11 497
50. Reich NC, Pfeffer LM (1990) Evidence for involvement of protein kinase C in the cellular response to interferon α. Proc Natl Acad Sci USA 87: 8761
51. Slaga TJ, Sivak AW, Boutwell RA (eds) (1978) Carcinogenesis: a comprehensive survey, vol. 2. Raven, New York
52. Slaga TJ, Fischer SM, Nelson K, Gleason GL (1980) Studies on the mechanism of skin tumor promotion: evidence for several stages in promotion. Proc Natl Acad Sci USA 77: 3659
53. Staehlin T, Hobbs DS, Kung H-F, Pestka S (1981) Purification of recombinant human leukocyte interferon (IFNrA) with nonoclonal antibodies. Methods Enzymol 78: 505
54. Su Z-Z, Fisher PB (1989) Early events in methyl methanesulfonate enhancement of adenovirus transformation of cloned rat embryo fibroblast cells. Mol Carcinogenesis 2: 252
55. Su Z-Z, Fisher PB (1992) Enhancement of oncogene-mediated transformation in cloned rat embryo fibroblast (CREF) cells by 3-aminobenzamide. In: Poirier GL, Moreau P (eds) ADP-ribosylation reactions. Springer, New York Berlin Heidelberg, pp 203–210
56. Su Z-Z, Grunberger D, Fisher PB (1991) Suppression of adenovirus type 5 E1A-mediated transformation and expression of the transformed phenotype by caffeic acid phenethyl ester (CAPE). Mol Carcinogenesis 4: 231
57. Unanue ER, Allen PM (1986) Comment on the finding of Ia expression in nonlymphoid cells. Lab Invest 55: 123
58. Wender PA, Konrad FK, Sharkey NA, Dell'Aquila ML, Blumberg PM (1986) Analysis of the phorbol ester pharmacophore on protein kinase C as a guide to the rational design of new classes of analogs. Proc Natl Acad Sci USA 83: 4214
59. Whitwell HL, Hughes HPA, Moore M, Ahmed A (1984) Expression of major histocompatibility antigens and leucocyte infiltration in benign and malignant human breast disease. Br J Cancer 49: 161
60. Yan C, Sehgal PB, Tamm I (1989) Signal transduction pathways in the induction of 2′,5′-oligoadenylate synthetase gene expression by interferon α/β. Proc Natl Acad Sci USA 86: 2243
61. Yap WH, Teo TS, Tan YH (1986) An early event in the interferon-induced transmembrane signalling process. Science 234: 355
62. Yap WH, Teo TS, McCoy E, Tan YH (1986) Rapid and transient rise in diacylglycerol concentration in Daudi cells exposed to interferon. Proc Natl Acad Sci USA 83: 7765