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European Journal of Pharmacology 345 (1998) 199–206

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Analysis of the expression of intercellular adhesion molecule-1 in cells of the human bronchial epithelial cell line NCI-H292

Yuhko Togashi [a], Kouya Yamaki [a], Takumi Sakai [a], Masako Watanabe-Kobayashi [a], Suetsugu Mue [b], Kazuo Ohuchi [a,*]

[a] *Department of Pathophysiological Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku University, Aoba Aramaki, Aoba-ku, Sendai, Miyagi 980-8578, Japan*

[b] *Department of Health and Welfare Science, Faculty of Physical Education, Sendai College, Shibata, Miyagi 989-1963, Japan*

Received 23 September 1997; revised 30 December 1997; accepted 6 January 1998

Abstract

The mechanism of the expression of intercellular adhesion molecule-1 (ICAM-1) on epithelial cells was analyzed using NCI-H292 cells, a human bronchial epithelial cell line. Treatment with interferon-γ (100 U/ml) or the protein kinase C activator 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (16.2 nM) induced ICAM-1 expression. The interferon-γ-induced ICAM-1 expression was reduced by the tyrosine kinase inhibitor genistein (4′,5,7-trihydroxyisoflavone) (37 to 185 μM), but not by the protein kinase C inhibitor Ro 31-8425 ((3-[8-(aminomethyl)-6,7,8,9-tetrahydropyrido [1,2-*a*]indol-10-yl]-4-(1-methyl-1*H*-pyrrole-2,3-dione) (10 μM). The TPA-induced ICAM-1 expression was reduced by the protein kinase C inhibitor Ro 31-8425 (1 to 10 μM), but not by the tyrosine kinase inhibitor genistein (185 μM). The protein kinase A inhibitor H-89 (*N*-[2-((*p*-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide) did not affect the ICAM-1 expression induced by interferon-γ or TPA. Pyrrolidine dithiocarbamate (1-pyrrolidinecarbodithioic acid) (100 μM), an inhibitor of nuclear factor κB (NF-κB) activation, enhanced the ICAM-1 expression induced by interferon-γ, but reduced that induced by TPA. The changes in ICAM-1 expression on the cell surface were correlated with the changes in ICAM-1 mRNA levels. Combined treatment with interferon-γ and TPA induced more than additive ICAM-1 expression. These findings suggest that interferon-γ induces ICAM-1 expression by a tyrosine kinase-dependent mechanism, but that TPA induces it by a protein kinase C- and NF-κB-dependent mechanism. © 1998 Elsevier Science B.V.

Keywords: ICAM-1 (intercellular adhesion molecule-1); Interferon-γ; TPA (12-*O*-tetradecanoylphorbol 13-acetate); Epithelial cell; NCI-H292; Tyrosine kinase; Protein kinase C; Nuclear factor κB

1. Introduction

Cell adhesion that is mediated by specific cell-surface molecules is important in establishing and maintaining inflammation (Springer, 1990), bronchial asthma (Wegner et al., 1990), rheumatoid arthritis (Chin et al., 1990), atopic dermatitis (Lawley and Kubota, 1991), tumor metastasis (Rice and Bevilacqua, 1989) and allograft rejection (Isobe et al., 1992). Intercellular adhesion molecule-1 (ICAM-1; CD54) is an 80- to 114-kDa integral membrane glycoprotein and a member of the immunoglobulin superfamily (Staunton et al., 1988). ICAM-1 serves as a ligand for lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18) (Rothlein et al., 1986; Marlin and Springer, 1987), Mac-1 (CD11b/CD18) (Diamond et al., 1991), and sialoglycoprotein CD43 (Rosenstein et al., 1991). ICAM-1 is constitutively expressed at low levels on a subpopulation of hematopoietic cells (Dustin et al., 1986), and various proinflammatory mediators, including interferon-γ, tumor necrosis factor-α, interleukin-1 (Pober et al., 1986; Rothlein et al., 1988), bacterial products lipopolysaccharides (Yu et al., 1986) and the protein kinase C activator phorbol ester, enhance ICAM-1 expression on many types of cell (Lane et al., 1989; Griffiths et al., 1990). Anti-ICAM-1 antibodies have been demonstrated to inhibit inflammatory responses in various animal models (Barton et al., 1989; Isobe et al., 1992; Cosimi et al., 1990), and ICAM-1-deficient mice show reduced inflammatory responses (Sligh et al., 1993). Therefore, up-regulation of ICAM-1 expression by inflammatory mediators is thought to be an important step in inflammatory responses. The relevance of the

* Corresponding author. Tel.: +81-22-217-6860; fax: +81-22-217-6859.

ICAM-1/β_2-integrin (LFA-1, Mac-1) mechanism for airway inflammation was demonstrated in a nonhuman primate model of asthma (Wegner et al., 1990). In this model, antigen inhalation induced augmented expression of ICAM-1 on bronchial epithelial cells and vascular endothelial cells, and a corresponding influx of eosinophils into the airway lumen. Furthermore, anti-ICAM-1 antibodies prevented the antigen-induced eosinophil influx and airway hyperreactivity (Wegner et al., 1990). An increase in ICAM-1 expression was also observed on bronchial epithelial cells cultured in the presence of cytokines (Look et al., 1992; Tosi et al., 1992b; Bloemen et al., 1993) and after viral infection (Tosi et al., 1992a; Pilewski et al., 1995), and anti-ICAM-1 antibodies inhibited adhesion of leukocytes to ICAM-1 expressing bronchial epithelial cells in culture (Look et al., 1992). These observations suggest that the increased expression of ICAM-1 in the airway enhances local inflammatory processes, and that ICAM-1 is one of the important target molecules for the therapy of bronchial asthma.

ICAM-1 expression on bronchial epithelial cells is selectively responsive to interferon-γ (Look et al., 1992; Ohh and Takei, 1994) but not to tumor necrosis factor-α or interleukin-1β, and the ICAM-1 level on cells of the human bronchial epithelial cell line NCI-H292 is also sensitive to interferon-γ and the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (Bloemen et al., 1993). In endothelial cells, ICAM-1 expression induced by interferon-γ is mediated by the activation of protein kinase C (Renkonen et al., 1990; Eissner et al., 1994). However, little is known about the intracellular signal transduction pathways leading to the increased ICAM-1 expression elicited by interferon-γ in bronchial epithelial cells. Therefore, the present investigation was intended to clarify pharmacologically the mechanism of induction of ICAM-1 expression by interferon-γ and TPA in NCI-H292 cells.

2. Materials and methods

2.1. Cell culture

NCI-H292 cells, a bronchial epithelial cell line (American Type Culture Collection CRL 1848, Rockvill, MD, USA), were cultured at 37°C in RPMI 1640 medium (Nissui Seiyaku, Tokyo, Japan) containing 10% fetal bovine serum (Flow Laboratories, McLean, VA, USA), penicillin G potassium (18 mg/ml) and streptomycin sulfate (50 mg/ml) (Meiji Seika, Tokyo, Japan) on uncoated culture flasks, passaged twice a week and used at passage 15. When NCI-H292 cells were almost confluent, they were harvested from the flask by treatment with phosphate-buffered saline (PBS, pH 7.4) containing 0.25% (w/v) trypsin (Flow Laboratories) and 0.01% (w/v) EDTA, and plated onto dishes and incubated in the same medium. When monolayers of NCI-H292 cells were loosely confluent, they were used for each experiment described below.

2.2. Treatment of NCI-H292 cells

Monolayers of NCI-H292 cells that were subconfluent in the dishes were incubated for 1 h at 37°C in RPMI 1640 medium containing 10% fetal bovine serum with or without drugs. Drugs used were recombinant human interferon-γ (Genzyme, Cambridge, MA, USA), TPA (Sigma, St. Louis, MO, USA), K-252a ($8R^*$, $9S^*$, $11S^*$)-(−)-9-hydroxy-9-methoxycarbonyl-8-methyl-2,3,9,10-tetrahydro-8,11-epoxy-1H, 8H, 11H-2, 7b, 11a-triazadibenzo-[a,g]cycloocta[a,d,e]-trinden-1-one) (Kyowa Medex, Tokyo, Japan), Ro 31-8425 (3-[8-(aminomethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl]-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,3-dione) (a gift from Dr. Kohji Yamada at Eisai, Tokyo, Japan), genistein (4′,5,7-trihydroxyisoflavone) (Sigma), H-89 (*N*-[2-((*p*-bromocinnamyl)-amino)ethyl]-5-isoquinolinesulfonamide) (Seikagaku Kogyo, Tokyo, Japan), pyrrolidine dithiocarbamate (1-pyrrolidinecarbodithioic acid) (Sigma). The protein kinase C activators teleocidin and aplysiatoxin (Fujiki et al., 1984) were gifts from Dr. Hirota Fujiki at Saitama Cancer Center Research Institute, Saitama, Japan. They were dissolved in ethanol and added to the medium. After a 1-h incubation, cells were further incubated for various periods in the medium containing TPA or interferon-γ and corresponding drug described above. The final concentration of ethanol in the medium was adjusted to 0.1% (v/v). After treatment with drugs, the viability of the cells was examined by the trypan blue exclusion test, and no cytotoxic effect was observed.

2.3. Measurement of ICAM-1 surface expression on NCI-H292 cells

After stimulation with interferon-γ or TPA in the presence or absence of drugs, the cells were harvested from the dishes by exposure to 0.25% trypsin–0.01% (w/v) EDTA in PBS followed by gentle scraping with a rubber policeman. The cells (5×10^6 cells) were then incubated in 120 μl of PBS containing anti-human ICAM-1 mAb BBA3 (1:600, R&D Systems, Abingdon, UK) and 0.5% (w/v) bovine serum albumin (Wako) at 4°C for 30 min. After being washed with PBS containing 0.5% (w/v) bovine serum albumin, the cells were incubated in 120 μl of PBS containing fluorescein isothiocianate-conjugated goat anti-mouse IgG (1:200, Seikagaku Kogyo) and 0.5% (w/v) bovine serum albumin at 4°C for 30 min. Subsequently, the cells were washed twice and 10 000 cells were analyzed for fluorescence intensity by flow cytometry, using FACScan (Becton Dickinson, San Jose, CA, USA). The background fluorescence was determined using cells incubated only with the secondary antibody. Flow cytometry data are depicted as histograms of the number of cells (y-axis) vs. fluorescence intensity (x-axis) on a log scale.

The mean ICAM-1-specific fluorescence intensity (MFI) of cells from various culture conditions was determined by subtracting the mean background cell fluorescence.

2.4. Semi-quantitation of ICAM-1 mRNA by reverse transcription–polymerase chain reaction

Total cellular RNA was isolated by the acid guanidinium thiocyanate-phenol–chloroform extraction method (Chomczynski and Sacchi, 1987). One microgram RNA from each sample was reverse transcribed at 37°C for 1 h in 20 μl of first strand buffer (50 mM Tris–HCl, 75 mM KCl, and 3 mM $MgCl_2$, pH 8.3) (GIBCO BRL, Gaithersburg, MD, USA) containing 3 μg of random hexamer oligonucleotides, 200 U of moloney murine leukemia virus reverse transcriptase (GIBCO BRL), 20 nmol of each deoxynucleotide triphosphate (dNTP) mixture, and 200 nmol of dithiothreitol. The cDNA samples were then amplified by polymerase chain reaction (PCR) with sense and antisense primers for ICAM-1 designed from the published sequences (Staunton et al., 1988). The sequences of primers used were (former) 5′-ACGTACCTCTATAACCGC-CAGC-3′, and (reverse) 5′-ATATGGGAAGGCCGAG-GAAGAG-3′, which amplify 143 ICAM-1 base pair (bp) fragments. PCR consisted of 5 μl of reverse transcribed RNA and 45 μl PCR buffer (10 mM Tris–HCl, 50 mM KCl, and 4 mM $MgCl_2$, pH 8.3) containing 1.0 pmol each primer, 1.0 nmol dNTPs, and 1.25 U recombinant Taq DNA polymerase (Takara Shuzo, Tokyo, Japan). Using a thermal cycler (GeneAmp PCR System 2400, Perkin-Elmer Cetus, Norwalk, CT, USA), PCR was performed for 28 cycles; 1 min denaturation at 95°C, 2 min annealing at 60°C, and 3 min extension at 72°C. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. The sequences of PCR primers used were; (former) 5′-TGATGACATCAAGAAGGTGGTGAAG-3′, and (reverse) 5′-TCCYTTGGAGGCCATGTAGGCCAT-3′, which amplify 249 GAPDH bp fragments, and PCR was performed for 22 cycles; 0.5 min denaturation at 94°C, 1 min annealing at 59°C, and 2 min extension at 72°C (Tanabe et al., 1995). The amplification product was fractionated by electrophoresis on a 1.5% agarose minigel and visualized by ethidium bromide staining. The levels of mRNA for ICAM-1 and GAPDH were quantified by scanning densitometry, and the ratio of ICAM-1 mRNA to GAPDH mRNA was calculated.

2.5. Statistical analysis

The difference in MFI between the corresponding two groups was analyzed for statistical significance by Student's *t*-test. In the densitometric analysis, the statistical significance of the difference in the density ratio of ICAM-1 mRNA to GAPDH mRNA between the corresponding two groups was also analyzed by Student's *t*-test.

3. Results

3.1. Effects of K-252a on interferon-γ- and TPA-induced ICAM-1 expression

ICAM-1 was expressed weakly on the surface of cells of the human epithelial cell line NCI-H292 in the unstimulated condition (Fig. 1). Incubation of the cells in the

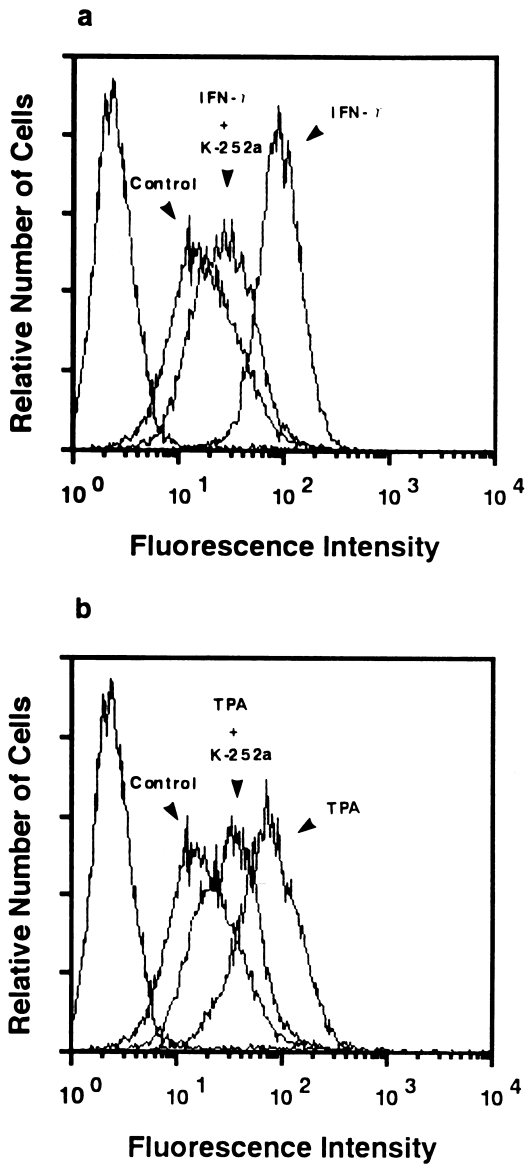

Fig. 1. Effects of K-252a on interferon-γ- and TPA-induced ICAM-1 expression. NCI-H292 cells were incubated at 37°C for 8 h in medium containing interferon-γ (IFN-γ) (100 U/ml) (a) or TPA (16.2 nM, 10 ng/ml) (b) in the presence or absence of K-252a (214 nM, 100 ng/ml). ICAM-1 expression was measured by indirect immunofluorescence and FACscan analysis as described in Section 2. Representative flow cytometry histograms for 10,000 cells showing background fluorescence (the left-side histograms) and specific surface ICAM-1 immunofluorescence. The mean MFI ± S.E.M.: (a) Control, 19.7 ± 0.9; IFN-γ 100 U/ml, 88.0 ± 6.2; IFN-γ 100 U/ml + K-252a 214 nM, 25.6 ± 1.2. (b) Control, 19.8 ± 1.0; TPA 16.2 nM, 80.7 ± 5.9; TPA 16.2 nM + K-252a 214 nM, 30.8 ± 1.9. Statistical significance: Control vs. IFN-γ or TPA, $P < 0.001$; IFN-γ vs. IFN-γ + K-252a or TPA + K-252a, $P < 0.001$.

medium containing interferon-γ (30, 100 and 300 U/ml) or TPA (4.9, 16.2 and 48.6 nM, 3, 10 and 30 ng/ml, respectively) for 8 h further enhanced the ICAM-1 expression in a concentration-dependent manner (the mean MFI ± S.E.M. from three assays: interferon-γ 0 U/ml, 19.4 ± 2.1; 30 U/ml, 60.3 ± 3.9; 100 U/ml, 86.3 ± 4.5 and 300 U/ml, 112.4 ± 6.1. TPA 0 nM, 19.7 ± 2.4; 4.9 nM, 39.1 ± 2.5; 16.2 nM, 50.7 ± 4.2 and 48.6 nM, 58.1 ± 4.1). In the following pharmacological experiments, 100 U/ml interferon-γ and 16.2 nM TPA were used to stimulate the expression of ICAM-1. To clarify the role of protein kinases in the up-regulation of ICAM-1 expression induced by interferon-γ or TPA, the effects of K-252a, a non-selective inhibitor of protein kinases, were examined. Incubation of the cells for 8 h in the presence of K-252a reduced the ICAM-1 expression induced by interferon-γ (100 U/ml) or TPA (16.2 nM) in a concentration-dependent manner. Fig. 1a and b show the inhibition by K-252a at a concentration of 214 nM (100 ng/ml) of interferon-γ- and TPA-induced ICAM-1 expression, respectively.

The percent inhibition of interferon-γ-induced ICAM-1 expression by K-252a at 21.4, 64.2 and 214 nM was 41.3 ± 2.5, 58.5 ± 3.3 and 91.4 ± 3.5, respectively (the means ± S.E.M. from three assays: K-252a 0 nM vs. 21.4, 64.2 or 214 nM, $P < 0.001$). The percent inhibition of TPA-induced ICAM-1 expression by K-252a at 21.4, 64.2 and 214 nM was 38.8 ± 1.5, 62.8 ± 2.3 and 93.8 ± 4.2, respectively (the means ± S.E.M. from three assays: K-252a 0 nM vs. 21.4, 64.2 or 214 nM, $P < 0.001$). At these concentrations of K-252a, the basal expression of ICAM-1 was not affected at all.

3.2. Effects of Ro 31-8425, genistein and H-89 on interferon-γ- and TPA-induced ICAM-1 expression

To determine the species of protein kinases that are associated with interferon-γ- and TPA-induced ICAM-1 expression, the effects of the more selective protein kinase C inhibitor Ro 31-8425, the tyrosine kinase inhibitor genistein, and the cyclic AMP-dependent protein kinase inhibitor H-89 were examined. Treatment with Ro 31-8425 at 10 μM for 8 h did not inhibit interferon-γ (100 U/ml)-induced ICAM-1 expression (Fig. 2a), but strongly inhibited TPA (16.2 nM)-induced ICAM-1 expression (the mean MFI ± S.E.M. from three assays: control, 19.3 ± 2.2; TPA 16.2 nM, 49.8 ± 3.2; TPA 16.2 nM + Ro 31-8425 10 μM, 24.5 ± 2.1. TPA vs. TPA + Ro 31-8425, $P < 0.001$) (Fig. 2b). TPA-induced ICAM-1 expression was inhibited by Ro 31-8425 in a concentration-dependent manner at 1, 3 and 10 μM. The percent inhibition of TPA-induced ICAM-1 expression by Ro 31-8425 at 1, 3 and 10 μM was 30.1 ± 1.1, 56.2 ± 3.1 and 83.0 ± 4.5, respectively (the means ± S.E.M. from three assays: TPA vs. TPA + Ro 31-8425 1, 3 or 10 μM, $P < 0.001$). In contrast, treatment with genistein at 185 μM (50 μg/ml) did not inhibit TPA-induced ICAM-1 expression (Fig. 3b), but inhibited

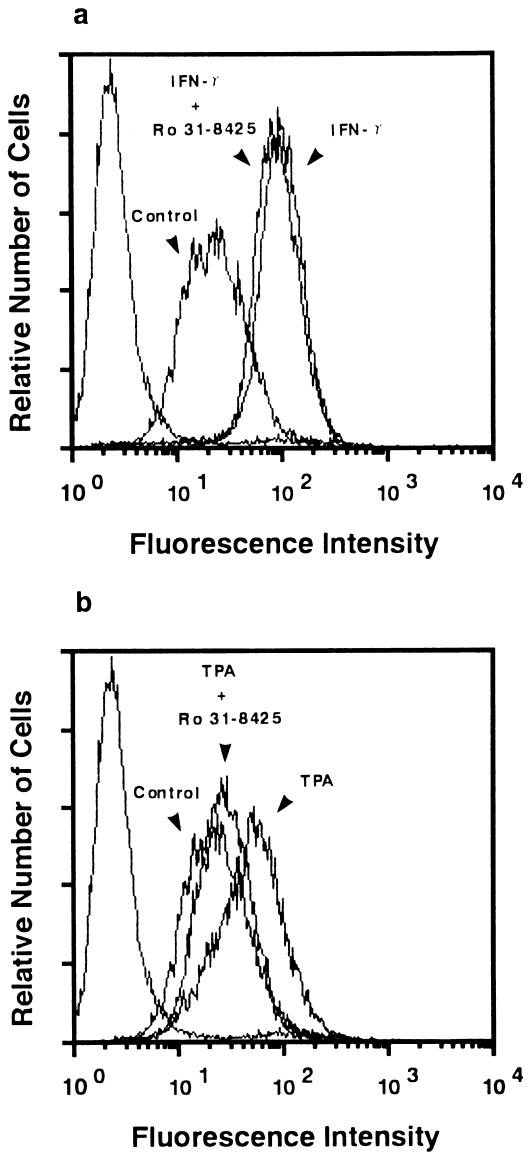

Fig. 2. Effects of Ro 31-8425 on interferon-γ- and TPA-induced ICAM-1 expression. NCI-H292 cells were incubated at 37°C for 8 h in medium containing interferon-γ (IFN-γ) (100 U/ml) (a) or TPA (16.2 nM, 10 ng/ml) (b) in the presence or absence of Ro 31-8425 (10 μM). ICAM-1 expression was measured by indirect immunofluorescence and FACscan analysis as described in Section 2. Representative flow cytometry histograms for 10,000 cells showing background fluorescence (the left-side histograms) and specific surface ICAM-1 immunofluorescence. The mean MFI ± S.E.M. from three assays: (a) Control, 19.3 ± 2.2; IFN-γ 100 U/ml, 86.6 ± 6.2; IFN-γ 100 U/ml + Ro 31-8425 10 μM, 91.7 ± 5.1. (b) Control, 19.3 ± 2.2; TPA 16.2 nM, 49.8 ± 3.2; TPA 16.2 nM + Ro 31-8425 10 μM, 24.5 ± 2.1. Statistical significance: Control vs. IFN-γ or IFN-γ + TPA, $P < 0.001$; Control vs. TPA or TPA + Ro 31-8425, $P < 0.001$; TPA vs. TPA + Ro 31-8425, $P < 0.001$.

interferon-γ-induced ICAM-1 expression (the mean MFI ± S.E.M. from three assays: control, 24.3 ± 2.2; interferon-γ 100 U/ml, 87.3 ± 4.1; interferon-γ 100 U/ml + genistein 185 μM, 43.0 ± 1.9; interferon-γ vs. interferon-γ + genistein, $P < 0.001$) (Fig. 3a). Interferon-γ-induced ICAM-1 expression was inhibited by genistein in a con-

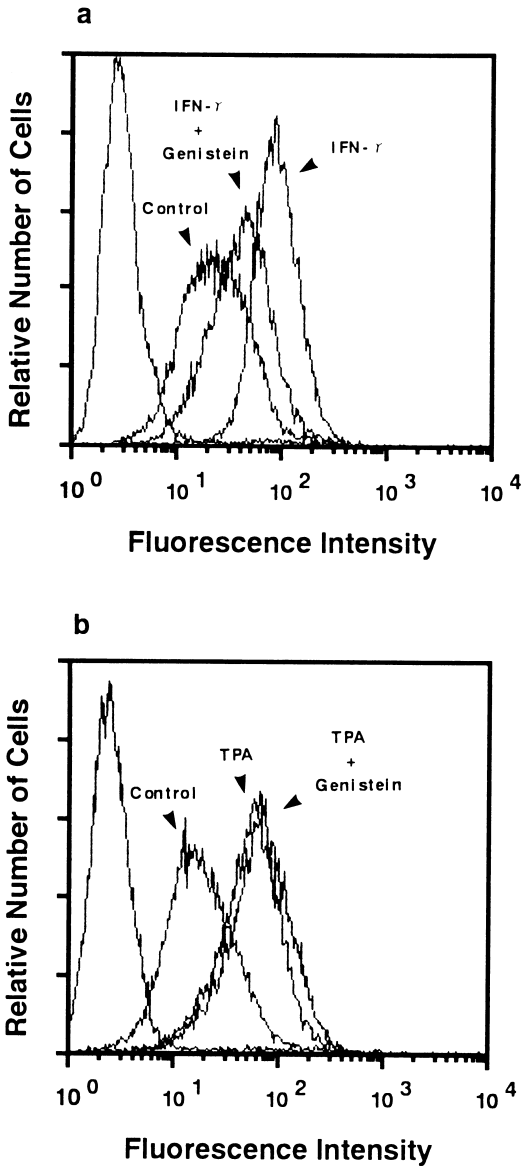

Fig. 3. Effects of genistein on interferon-γ- and TPA-induced ICAM-1 expression. NCI-H292 cells were incubated at 37°C for 8 h in medium containing interferon-γ (IFN-γ) (100 U/ml) (a) or TPA (16.2 nM, 10 ng/ml) (b) in the presence or absence of genistein (185 μM, 50 μg/ml). ICAM-1 expression was measured by indirect immunofluorescence and FACscan analysis as described in Section 2. Representative flow cytometry histograms for 10,000 cells showing background fluorescence (the left-side histograms) and specific surface ICAM-1 immunofluorescence. The mean MFI ± S.E.M. from three assays: (a) Control, 24.3 ± 1.2; IFN-γ 100 U/ml, 87.3 ± 5.1; IFN-γ 100 U/ml + genistein 185 μM, 43.0 ± 2.5. (b) Control, 19.7 ± 2.1; TPA 16.2 nM, 55.1 ± 4.1; TPA 16.2 nM + genistein 185 μM, 62.5 ± 5.3. Statistical significance: Control vs. IFN-γ or IFN-γ + genistein, $P < 0.001$; Control vs. TPA or TPA + genistein, $P < 0.001$; IFN-γ vs. IFN-γ + genistein, $P < 0.001$.

centration-dependent manner at 37, 111 and 185 μM. The percent inhibition of interferon-γ-induced ICAM-1 expression by genistein at 37, 111 and 185 μM was 23.4 ± 1.2, 44.6 ± 2.5 and 70.3 ± 4.1, respectively (interferon-γ vs. interferon-γ + genistein 37, 111 or 185 μM, $P < 0.001$). Treatment with H-89 did not inhibit either interferon-γ- or TPA-induced ICAM-1 expression at concentrations up to 2.24 μM (1.0 μg/ml) (data not shown). Neither inhibitor at these concentrations affected the basal expression of ICAM-1 (data not shown).

3.3. Effects of pyrrolidine dithiocarbamate on interferon-γ- and TPA-induced ICAM-1 expression

To clarify the role of NF-κB activation in the expression of ICAM-1 induced by interferon-γ or TPA, the effects of pyrrolidine dithiocarbamate, a potent inhibitor of NF-κB activation, were examined. As shown in Fig. 4a, interferon-γ (100 U/ml)-induced ICAM-1 expression at 8 h was enhanced by pyrrolidine dithiocarbamate at 100 μM (the mean MFI ± S.E.M. from three assays: interferon-γ 0 U/ml, 26.6 ± 1.2; interferon-γ 100 U/ml, 83.2 ± 4.6; interferon-γ 100 U/ml + pyrrolidine dithiocarbamate 100 μM, 127.0 ± 6.1. interferon-γ vs. interferon-γ + pyrrolidine dithiocarbamate, $P < 0.001$); however, pyrrolidine dithiocarbamate at 30 μM showed no significant effect (data not shown). In contrast, TPA (16.2 nM)-induced ICAM-1 expression at 8 h was markedly suppressed by pyrrolidine dithiocarbamate at 100 μM (Fig. 4b) and 30 μM (the mean MFI ± S.E.M. from 3 assays: TPA 0 nM, 23.4 ± 1.2; TPA 16.2 nM, 47.8 ± 2.1; TPA 16.2 nM + pyrrolidine dithiocarbamate 30 μM, 39.1 ± 1.5; TPA 16.2 nM + dithiocarbamate 100 μM, 26.2 ± 1.5. TPA vs. TPA + pyrrolidine dithiocarbamate, $P < 0.001$). Pyrrolidine dithiocarbamate at 100 μM did not affect the basal expression of ICAM-1.

Reverse transcription–polymerase chain reaction (RT-PCR) revealed that the level of ICAM-1 mRNA in the cells was increased by treatment with interferon-γ (100 U/ml) or TPA (16.2 nM) for 4 h (Fig. 5). Treatment with pyrrolidine dithiocarbamate at 100 μM enhanced the interferon-γ-induced increase in the level of ICAM-1 mRNA, but reduced TPA-induced increase in the level of ICAM-1 mRNA (Fig. 5). In contrast, the level of GAPDH mRNA was not affected by these drugs (Fig. 5).

3.4. Synergistic effects of interferon-γ and TPA on ICAM-1 expression

Incubation of the cells for 8 h in medium containing interferon-γ (100 U/ml) or TPA (16.2 nM) increased the expression of ICAM-1 (Fig. 6). The combined treatment of the cells with interferon-γ (100 U/ml) and TPA (16.2 nM) for 8 h enhanced the ICAM-1 expression more than additively, i.e., synergistically (Fig. 6). The mean specific fluorescence intensity (MFI) for ICAM-1 was increased 3.6- and 2.1-fold by treatment with interferon-γ (100 μM) and TPA (16.2 nM), respectively, and the combined treatment with interferon-γ and TPA resulted in a 9.1-fold increase in ICAM-1 expression as compared to the control (the mean MFI ± S.E.M. from four assays: control, 24.3 ±

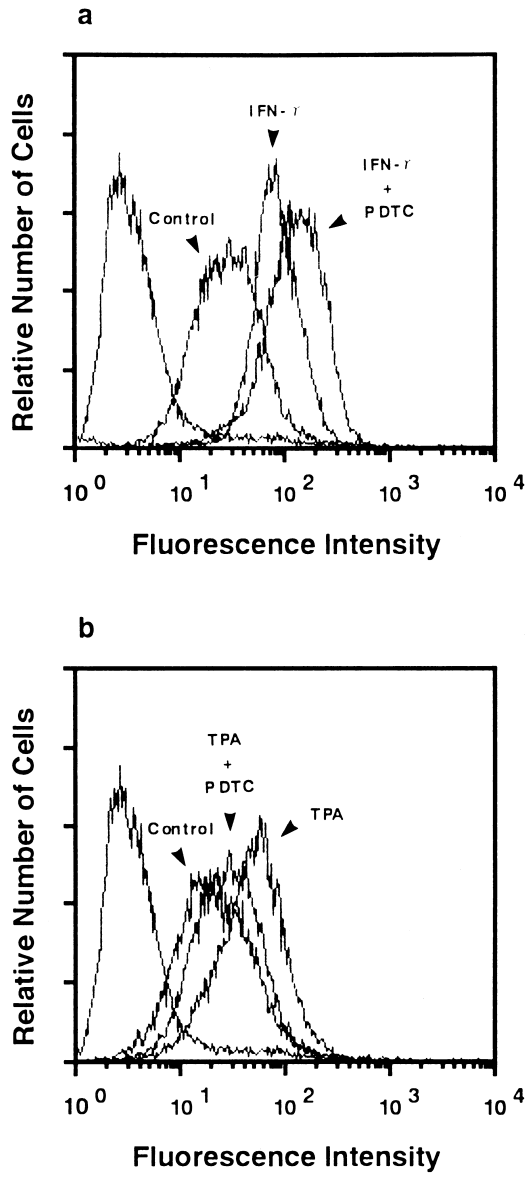

Fig. 4. Effects of pyrrolidine dithiocarbamate on interferon-γ- and TPA-induced ICAM-1 expression. NCI-H292 cells were incubated at 37°C for 8 h in medium containing interferon-γ (IFN-γ) (100 U/ml) (a) or TPA (16.2 nM, 10 ng/ml) (b) in the presence or absence of pyrrolidine dithiocarbamate (PDTC) (100 μM, 16.4 μg/ml). The ICAM-1 expression was measured by indirect immunofluorescence and FACscan analysis as described in Section 2. Representative flow cytometry histograms for 10,000 cells showing background fluorescence (the left-side histograms) and specific surface ICAM-1 immunofluorescence. The mean MFI ± S.E.M. from three assays: (a) Control, 23.6 ± 1.8; IFN-γ 100 U/ml, 83.2 ± 4.9; IFN-γ 100 U/ml + PDTC 100 μM, 127.0 ± 8.9. (b) Control, 21.4 ± 2.5; TPA 16.2 nM, 47.8 ± 3.8; TPA 16.2 nM + PDTC 100 μM, 26.6 ± 1.8. Statistical significance: Control vs. IFN-γ or IFN-γ + PDTC, $P < 0.001$; Control vs. TPA or TPA + PDTC, $P < 0.001$; IFN-γ vs. IFN-γ + PDTC, $P < 0.001$; TPA vs. TPA + PDTC, $P < 0.001$.

2.0; TPA 16.2 nM, 57.3 ± 7.1; interferon-γ 100 U/ml, 86.9 ± 5.5; interferon-γ + TPA 16.2 nM, 223.3 ± 15.6; interferon-γ or TPA vs. interferon-γ + TPA, $P < 0.001$). Treatment with interferon-γ and other protein kinase C activators such as teleocidin (2.25 nM, 10 ng/ml) and

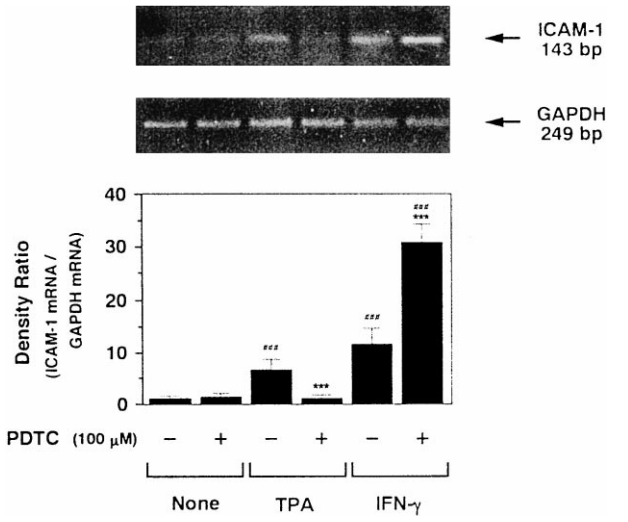

Fig. 5. Effects of pyrrolidine dithiocarbamate on the level of ICAM-1 mRNA in NCI-H292 cells stimulated with interferon-γ and TPA. NCI-H292 cells were incubated at 37°C for 4 h in medium containing interferon-γ (IFN-γ) (100 U/ml) or TPA (16.2 nM, 10 ng/ml) in the presence or absence of pyrrolidine dithiocarbamate (PDTC) (100 μM, 16.4 μg/ml). Total RNA was extracted and RT-PCR was performed as described in Section 2. The levels of mRNA for ICAM-1 and GAPDH were quantitated by scanning densitometry, and the density ratio of ICAM-1 mRNA to GAPDH mRNA was calculated. To facilitate comparison, the ratio obtained for the cells incubated for 4 h in the medium without drugs was set as 1.0. Values are the means from three independent experiments with S.E.M. shown by vertical bars. Statistical significance: $^{***}P < 0.001$ vs. corresponding control. $^{\#\#\#}P < 0.001$ vs. None without PDTC.

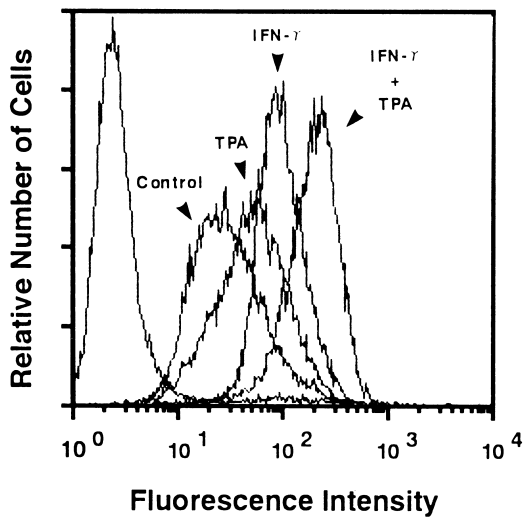

Fig. 6. Effects of combined treatment with TPA and interferon-γ on ICAM-1 expression. NCI-H292 cells were incubated at 37°C for 8 h in medium containing interferon-γ (IFN-γ) (100 U/ml), TPA (16.2 nM, 10 ng/ml), or both. ICAM-1 expression was measured by indirect immunofluorescence and FACscan analysis as described in Section 2. Representative flow cytometry histograms for 10,000 cells showing background fluorescence (the left-side histogram) and specific surface ICAM-1 immunofluorescence. The mean MFI ± S.E.M. from four assays: Control, 24.3 ± 2.0; TPA 16.2 nM, 57.3 ± 7.1; IFN-γ 100 U/ml, 86.9 ± 5.5; TPA 16.2 nM + IFN-γ 100 U/ml, 223.3 ± 15.6. Statistical significance: Control vs. TPA, IFN-γ or IFN-γ + TPA, $P < 0.001$; IFN-γ + TPA vs. IFN-γ or TPA, $P < 0.001$.

aplysiatoxin (14.9 nM, 10 ng/ml) also had a synergistic effect on ICAM-1 expression (fold increase of MFI at 8 h, the means ± S.E.M. from three assays: teleocidin, 2.5 ± 0.1; interferon-γ, 3.5 ± 0.2; teleocidin + interferon-γ, 9.6 ± 0.5; aplysiatoxin, 2.3 ± 0.1; interferon-γ, 3.4 ± 0.1; aplysiatoxin + interferon-γ, 8.9 ± 0.4; teleocidine or interferon-γ vs. teleocidine + interferon-γ, aplysiatoxin or interferon-γ vs. aplysiatoxin + interferon-γ, $P < 0.001$).

4. Discussion

Using cells of the human epithelial cell line NCI-H292, Bloemen et al. (1993) reported that the expression of ICAM-1 is increased by stimulation with interferon-γ or TPA, but not by tumor necrosis factor-α or interleukin-1β. However, little is known about the intracellular regulatory mechanisms which trigger ICAM-1 up-regulation by interferon-γ and TPA in NCI-H292 cells. Therefore, the present investigation was performed to clarify pharmacologically the signal transduction pathways involved in the ICAM-1 expression induced by interferon-γ or TPA on cells of the human epithelial cell line NCI-H292.

Ohh and Takei (1994) reported that up-regulation of the level of ICAM-1 mRNA by interferon-γ or TPA in murine fibroblast Ltk$^-$-cells is due to the stabilization of ICAM-1 mRNA, and that two distinct regions of ICAM-1 mRNA regulate its stability, one of which encodes the cytoplasmic domain and is responsive to interferon-γ, and the other which is in the 3′-untranslated region and is responsive to TPA. Therefore, the present results suggested that up-regulation of ICAM-1 expression by interferon-γ on NCI-H292 cells is due to the activation of tyrosine kinase, which influences the cytoplasmic domain of ICAM-1 mRNA by stabilizing it. Also, it was suggested that TPA up-regulates ICAM-1 expression by the activation of protein kinase C, which influences the 3′-untranslated region of ICAM-1 mRNA by stabilizing it.

Sequence analysis of the 5′ flanking region of the ICAM-1 gene revealed the presence of several potential regulatory elements. The human ICAM-1 gene contains a sequence at −540 that is recognized by the NF-κB/rel family of transcription factors (Voraberger et al., 1991). The sequence that resembles the NF-κB binding motif lies at −540, and NF-κB is thought to play a role in the expression of the ICAM-1 gene (Moynagh et al., 1994; Chen et al., 1995; Kawai et al., 1995). NF-κB activation occurs rapidly upon stimulation of cells with tumor necrosis factor-α, lipopolysaccharide or TPA (Baeuerle and Henkel, 1994). Therefore, we examined the role of NF-κB protein in the interferon-γ-induced activation of ICAM-1 gene in NCI-H292 cells by using pyrrolidine dithiocarbamate, an anti-oxidant inhibitor of NF-κB activation (Schreck et al., 1992). As expected, the TPA-induced ICAM-1 expression was reduced by 100 μM of pyrrolidine dithiocarbamate. However, the interferon-γ-induced ICAM-1 expression was enhanced by 100 μM of pyrrolidine dithiocarbamate (Fig. 4). The enhancement was not observed at 10 and 30 μM of pyrrolidine dithiocarbamate (data not shown). The contradictory effect of pyrrolidine dithiocarbamate on ICAM-1 expression was confirmed by RT-PCR of ICAM-1 mRNA, viz. pyrrolidine dithiocarbamate reduced the TPA-induced increase in the level of ICAM-1 mRNA, and up-regulated the interferon-γ-induced increase in the level of ICAM-1 mRNA. These findings suggested that the mechanism of the induction of ICAM-1 expression by interferon-γ is different from that for TPA. Pyrrolidine dithiocarbamate has been reported to increase the activity of the transcription factor AP-1 due to its antioxidant effect (Schenk et al., 1994), and the ICAM-1 gene contains a sequence that is recognized by AP-1 (Voraberger et al., 1991). Therefore, in NCI-H292 cells, AP-1 activation by pyrrolidine dithiocarbamate might further increase the expression of ICAM-1.

In this study, we showed that in NCI-H292 cells, interferon-γ induces ICAM-1 expression without mediating the activation of protein kinase C, and the activation of tyrosine kinase is crucial for the expression of ICAM-1. Furthermore, inhibition of NF-κB does not reduce the interferon-γ-induced ICAM-1 expression. In contrast, in endothelial cells, Renkonen et al. (1990) and Eissner et al. (1994) reported that the activation of protein kinase C is crucial in the expression of ICAM-1 induced by interferon-γ. Therefore, our findings indicate that the mechanism of ICAM-1 expression by interferon-γ in epithelial cells is quite different from that in endothelial cells.

5. Conclusion

Interferon-γ induces ICAM-1 expression on cells of the human epithelial cell line NCI-H292 by tyrosine kinase activation, without involvement of protein kinase C and cyclic AMP-dependent protein kinase, whereas TPA induces ICAM-1 expression through protein kinase C activation, without involvement of tyrosine kinase and cyclic AMP-dependent protein kinase A. Furthermore, pyrrolidine dithiocarbamate inhibits TPA-induced ICAM-1 expression but enhances interferon-γ-induced ICAM-1 expression.

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