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Retinoic Acid Induces Intercellular Adhesion Molecule-1 Hyperexpression in Human Thyroid Carcinoma Cell Lines*

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ABSTRACT

The expression of intercellular adhesion molecule-1 (ICAM-1) in tumoral tissues may promote their interaction with the immune system and cytotoxic effect on tumoral cells. This observation led to the investigation of ICAM-1 expression and modulation in different tumoral cell systems *in vitro*. Recently, retinoic acid-responsive elements have been found in the 5'-regulatory region of the human ICAM-1 gene. In the present study, we investigated, by flow cytometry, the effect of retinoic acid on the surface expression of ICAM-1 in human thyroid carcinoma cell lines. Two papillary (NPA and TPC-1), one follicular (WRO), one anaplastic (ARO) and one immortalized fetal (TAD-2) cell line have been studied. All of them produced constitutively ICAM-1; its surface expression and specific messenger ribonucleic acid (mRNA) levels were increased significantly by retinoic acid in all except the WRO cell line. ICAM-1 hyperexpression by retinoic acid was time dependent, reversible, and dependent on mRNA and protein synthesis. Furthermore, cytokines, such as interferon-γ and tumor necrosis factor-α, both individually and, to a greater extent, in combination with retinoic acid, increased ICAM-1 surface expression and its mRNA levels. In conclusion, retinoic acid is able to induce ICAM-1 up-regulation via mRNA accumulation in human thyroid carcinoma cell lines. (*J Clin Endocrinol Metab* **80:** 1129–1135, 1995)

INTERCELLULAR adhesion molecule-1 (ICAM-1) is a cell surface glycoprotein and a member of the immunoglobulin supergene family. Its expression is regulated in a wide variety of cells by various proinflammatory cytokines, such as interleukin-1, interferon-γ (IFNγ), and tumor necrosis factor-α (TNFα) (1). ICAM-1 represents the counterreceptor of β_2-integrins, such as lymphocyte function-associated antigen-1 (LFA-1/CD11a) and Vc_{3bi} complement receptor (MAC-1). The nonantigen-specific LFA-1/ICAM-1 adhesion system, with the CD-2/LFA-3 system, is considered to be crucial in the adhesion between lymphocytes and antigen-presenting cells, endothelial cells, or target cells (2). Recently, other counterreceptors of LFA-1, such as ICAM-2 (3) and ICAM-3 (4), have been identified. Many important immunological functions, such as major histocompatibility complex (MHC)-restricted and non-MHC-restricted cytotoxicity, interactions of T-helper lymphocytes with B-lymphocytes or antigen-presenting cells, and lymphocyte trafficking (5), are dependent on LFA-1/ICAM-1 interaction. Furthermore, ICAM-1 is regarded as the major rhinovirus receptor (6). Recent studies have shown that ICAM-1 is absent in normal thyroid cells and is expressed *de novo* in human thyroid carcinomas (7) and in autoimmune diseases such as Hashimoto's thyroiditis, whereas conflicting results have been obtained in Graves' disease (7–9). Moreover, up-regulation of ICAM-1 by cytokines is able to increase *in vitro* cluster formation of murine and human thyroid cells with murine splenocytes and human activated peripheral blood lymphocytes, respectively (10–13). To gain insight into the regulation of ICAM-1, we examined the effects of retinoic acid on the expression of ICAM-1 in cultured thyroid carcinoma cells.

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Materials and Methods

Human thyroid cell lines

Papillary NPA, follicular WRO, and anaplastic ARO cell lines were kindly provided by Dr. G. J. F. Juillard, University of California-Los Angeles. Papillary TPC-1 cell line was donated by Dr. M. Nagao, National Cancer Center (Tokyo, Japan). Cell lines were obtained by cloning primary cell cultures from cancer patients. The fetal cell line TAD-2, obtained from primary fetal cells and immortalized with the large T-antigen of simian virus-40 transfection, was provided by Dr. T. F. Davies, Mount Sinai (New York, NY).

NPA, WRO, and TAD-2 cell lines were cultured in RPMI-1640 (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (Gibco) and 2 mmol/L L-glutamine (Sigma Chemical Co., St. Louis, MO); TPC-1 and ARO were cultured in Dulbecco's Modified Eagle's Medium (Gibco) supplemented with 10% heat-inactivated fetal calf serum and 2 mmol/L L-glutamine.

To preserve the trypsin-sensitive ICAM-1 antigen, cells were detached at 37 C with PBS, 10 mmol/L HEPES, and 3 mg/mL EDTA (pH 7.4; all chemicals from Sigma Chemical Co., St. Louis, MO).

Chemicals

All-*trans*-retinoic acid (RA), dimethylsulfoxide (DMSO), actinomycin-D mannitol, and cycloheximide were purchased from Sigma. RA was dissolved in DMSO to form a 50 mmol/L stock solution and stored at −20 C. Control cell cultures received the same DMSO concentration (0.02%, final) as RA-treated cell cultures. Plastic dishes were wrapped in tinfoil to prevent light degradation of RA or actinomycin-D. Medium was changed every other day. Human recombinant IFNγ was purchased from Hoffman LaRoche (Nutley, NJ); human recombinant TNFα was obtained from PeproTech (Rocky Hill, NJ).

Monoclonal antibodies

Purified anti ICAM-1 (84H10) monoclonal antibody (mAb) was purchased from Immunotech (Marseille, France); purified antihuman leukocyte antigen (HLA) class I (W6/32) mAb was obtained from SeraLab (Crawley Down, United Kingdom); phycoerythrin (PE)-conjugated anti-HLA class II locus DR (OKDR) mAb and fluorescein (FITC)-conjugated anti-LFA-1/CD11a were purchased from Ortho Pharmaceuticals (Raritan, NJ). PE-conjugated anti-CD56/NCAM mAb was obtained from Becton Dickinson, Immunocytometry System (Mountain View, CA).

Purified anti-α_4-chain (B5G10) mAb of the β_1-family of integrins was kindly provided by Dr. M. E. Hemler (Boston, MA). Purified anti-ICAM-2 (6D5), ICAM-3 (CBR-IC3/1), and LFA-3 (BRIC 5) mAbs were obtained from material submitted to the nonlineage panel of the Fifth International Workshop and Conference on Human Leukocyte Differentiation Antigens. FITC-conjugated goat antimouse immunoglobulin G (IgG) was obtained from Ortho. Purified PE- and FITC-conjugated control (G1Cl) mAbs of the same isotype of specific mAbs were purchased from Becton Dickinson.

Immunofluorescence flow cytometry

Cells (1×10^5) were resuspended in 50 μL PBS and 0.5% BSA, 5 μL of each mAb were added, and tubes were incubated for 40 min at 4 C. The pellet was washed twice and resuspended in 30 μL of a 1:30 dilution of FITC-conjugated goat antimouse IgG for 30 min at 4 C. After two more washes, the cellular pellet was resuspended in 500 μL sheat fluid (Becton Dickinson). Samples were analyzed for cell surface fluorescence with a FACscan flow cytometer (Becton Dickinson). Results are expressed as the mean fluorescence intensity (MFI) in arbitrary units on a 4-decade logarithmic scale. The MFI of control Abs was set at 5 arbitrary units. Gates were set such that 5% of control cells, stained with isotype-matched mouse Ig, were positive.

Cell cycle analysis

After 72 h of RA treatment, cells were harvested, washed in PBS, and fixed in 70% ethanol. About 1×10^6 cells were incubated in PBS, 50 μg/mL propidium iodide, and 100 U/mL ribonuclease (Becton Dickinson) for 30 min at room temperature. Samples were analyzed for cell fluorescence with a FACscan flow cytometer (Becton Dickinson). The Cell Fit program (Becton Dickinson) was used to analyze the distribution of cells in the different phases of the cell cycle.

Northern blot

Total ribonucleic acid (RNA) was extracted by using the acid guanidinium thyocianate-phenol-chloroform extraction (14). Fifteen micrograms of RNA were analyzed by electrophoresis in agarose gel. RNA was transferred to nylon filter (Hybond-N, Amersham, Amersham, United Kingdom) and hybridized with a specific ICAM-1 complementary DNA (pG4H1.1) (15) kindly provided by Dr. T. A. Springer, Center for Blood Research, (Boston, MA) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe; both probes were ^{32}P (Amersham, Arlington, IL) labeled by the random primed method (Boehringer Mannheim, Mannheim, Germany). Nylon filters were exposed with a Kodak XAR autoradiographic film (Eastman Kodak, Rochester, NY) at −80 C. The magnitude of hybridization was quantified by scanning densitometry, using a computerized program (Howtek scan master 3 densitometer with RFLprint software, Pharmacia, Uppsala, Sweden). The values of ICAM-1 messenger RNA (mRNA) were normalized to the level of GAPDH mRNA.

Statistical analysis

Experimental data, where indicated, are presented as the mean ± SE from three to five separate experiments. Student's t test for paired samples was performed. Differences with $P < 0.05$ were considered statistically significant.

Results

Expression and modulation by RA of cellular adhesion molecules (CAM), MHC class I and DR molecules on thyroid cell lines

Immunofluorescence flow cytometry showed that all five cell lines were ICAM-1, MHC class I, and LFA-3 positive; only NPA was DR positive (Fig. 1). LFA-1/CD11a, ICAM-2, and ICAM-3 were not detectable.

After 72 h of culture, all cell lines, with exception of WRO, in the presence of 10 μmol/L (3 μg/mL) RA, a concentration known to stimulate ICAM-1 expression (16, 17), showed an

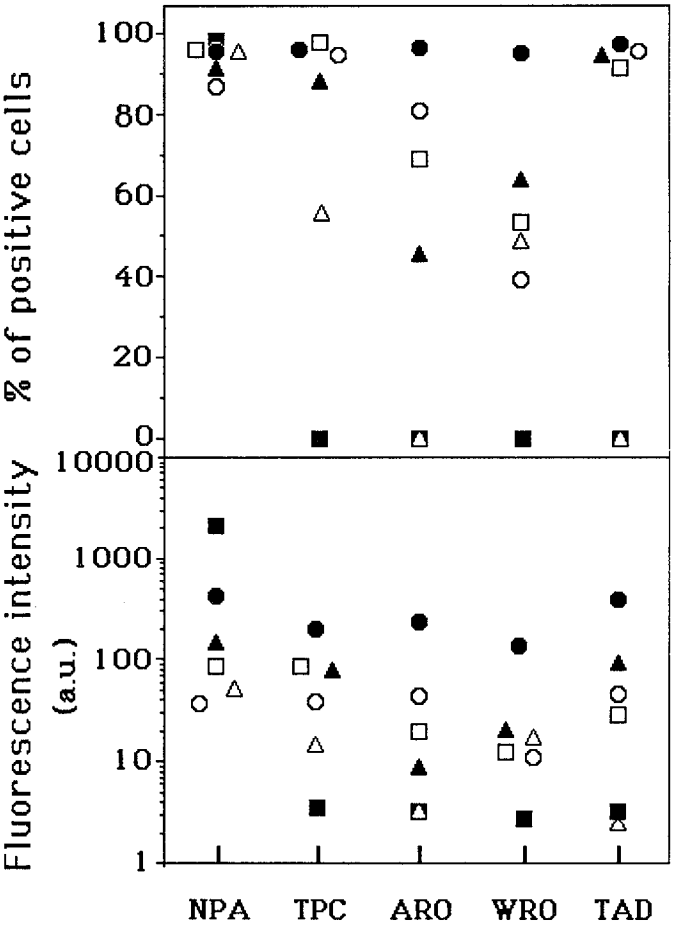

FIG. 1. Expression of CAMs on thyroid cell lines. Cells were incubated with specific monoclonal antibodies and analyzed by immunofluorescence flow cytometry. *Upper panel*, Percentage of positive cells; *lower panel*, MFI in arbitrary units. The percentage of cells stained by isotype-matched mouse Ig and the mean MFI values were lower than 5% and 5 arbitrary units, respectively. □, ICAM-1; △, α_4; ○, LFA-3; ▲, CD-56; ●, MHC-I; ■, DR.

increase in ICAM-1 expression compared to that in control cultures. The increase was 2.5-fold in NPA and TPC-1, and 1.5- and 2-fold in ARO and TAD-2 cell lines, respectively (Table 1). In contrast with the other three RA-responsive cell lines, only 20–30% of TAD-2 cells were RA responsive (Fig. 2).

ICAM-1 enhancement by RA treatment was unique. Other CAMs, constitutively expressed in our cells (Fig. 1), such as neural cell adhesion molecule (N-CAM/CD56), the α_4-chain of the β_1-family of integrins, and LFA-3, were unmodified by RA treatment.

RA was able to slightly increase (1.3-fold) the expression of DR molecule only in NPA cells, whereas it was ineffective in the other cell lines tested, which did not constitutively express this molecule. Only ARO cells of five cell lines showed a significant increase in MHC class I expression (Table 1).

To further clarify the role of RA in ICAM-1 modulation, a 24-h dose-response curve was performed in RA-sensitive NPA and TPC-1 and RA-insensitive WRO cell lines. Different concentrations of RA, ranging from 1 nmol/L to 50 μmol/L (0.3 ng/mL; 15 μg/mL), were used. In NPA, the increase in RA concentrations produced a parallel increase in ICAM-1 expression. The maximal effect was obtained at 10 μmol/L; higher doses were ineffective to further increase ICAM-1 expression. Similar results were obtained in the TPC-1 cell line, but higher RA concentrations (25 and 50 μmol/L) induced a paradoxical decrease in ICAM-1 expression. No change in ICAM-1 expression was observed in the WRO cell line even at higher doses of RA (Fig. 3). Cell viability, as assessed by propidium iodide exclusion staining, was not modified by all RA concentrations used.

A time-course experiment showed that the effect of RA in the RA-sensitive NPA cell line was time dependent and reversible. After 8, 12, and 24 h of RA stimulation, ICAM-1 expression increased from 1.25-fold to 1.5- and 1.9-fold, respectively, compared to the basal levels of the molecule. In NPA cells, after 24 h of incubation with 10 μmol/L RA, followed by incubation in RA-free medium, ICAM-1 hyper-

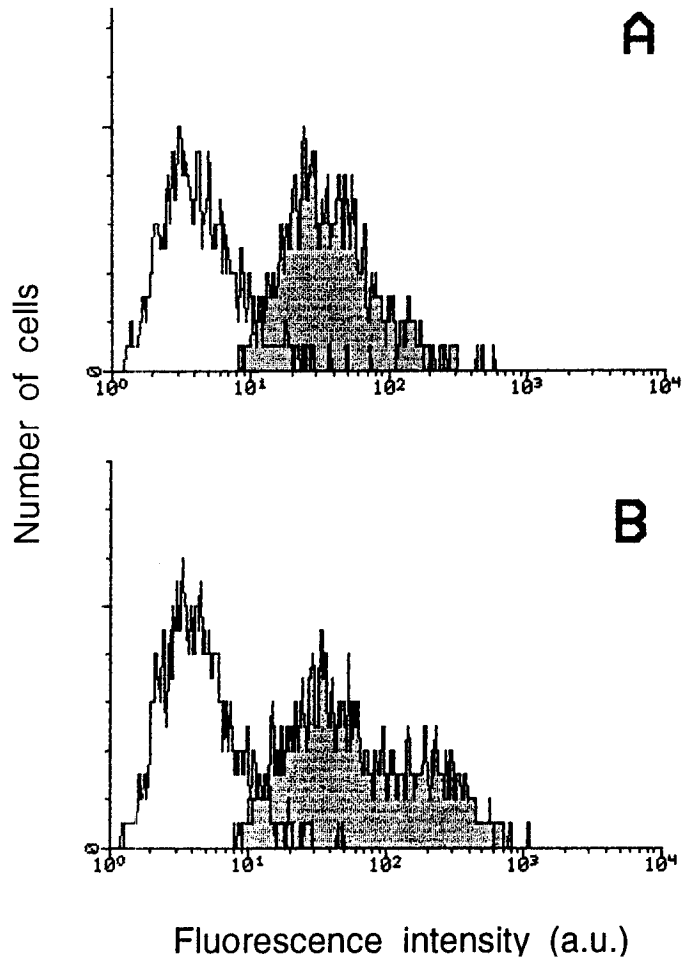

FIG. 2. Indirect immunofluorescence analysis of ICAM-1 expression in TAD-2 cells. Untreated cells (A) or cells treated with RA (B) (10^{-5} mol/L; 3 μg/mL) were cultured for 72 h. Then, cells were harvested and incubated with anti-ICAM-1 mAb and analyzed by immunofluorescence flow cytometry. The *empty histogram and gray histogram* show isotype-matched mouse Ig and anti-ICAM-1 mAb, respectively.

TABLE 1. Expression of ICAM-1, MHC-I, and DR molecule after 10^{-5} mol/L RA treatment in cultured human TAD-2 and thyroid carcinoma cells

RA	ICAM-1	%	MHC-I	%	DR	%
NPA						
−	91.0 ± 3.6	96 ± 0.7	420.3 ± 27	98 ± 0.6	2220 ± 28	99 ± 0.1
+	210.5 ± 22.1[a]	96 ± 1.0	415.1 ± 56	98 ± 0.3	2942 ± 108[a]	99 ± 0.3
TPC						
−	78.7 ± 2.5	98 ± 0.3	203.1 ± 35	97 ± 0.7	ND	
+	182.0 ± 11.6[a]	99 ± 0.6	209.6 ± 79	95 ± 0.2	ND	
TAD						
−	34.2 ± 5.0	92 ± 1.2	446.0 ± 20	97 ± 1.1	ND	
+	64.0 ± 12.0[a]	93 ± 2.4	493.0 ± 76	99 ± 0.2	ND	
ARO						
−	21.8 ± 2.6	72 ± 3.1	252.1 ± 10.5	96 ± 0.8	ND	
+	32.3 ± 4.4[a]	86 ± 7.0[a]	449 ± 5.5[b]	96 ± 1.1	ND	
WRO						
−	15.2 ± 3.0	53 ± 6.9	150.6 ± 24.1	95 ± 2.6	ND	
+	15.3 ± 4.8	53 ± 11	175.2 ± 39.9	97 ± 2.9	ND	

Cells were incubated with RA for 72 h at 37 C, harvested, incubated with the indicated monoclonal antibodies, and analyzed by flow cytometry. Each point represents the mean MFI ± SE of five independent experiments. ND, Not detectable. %, Percentage of positive cells.

[a] $P < 0.05$.

[b] $P < 0.01$.

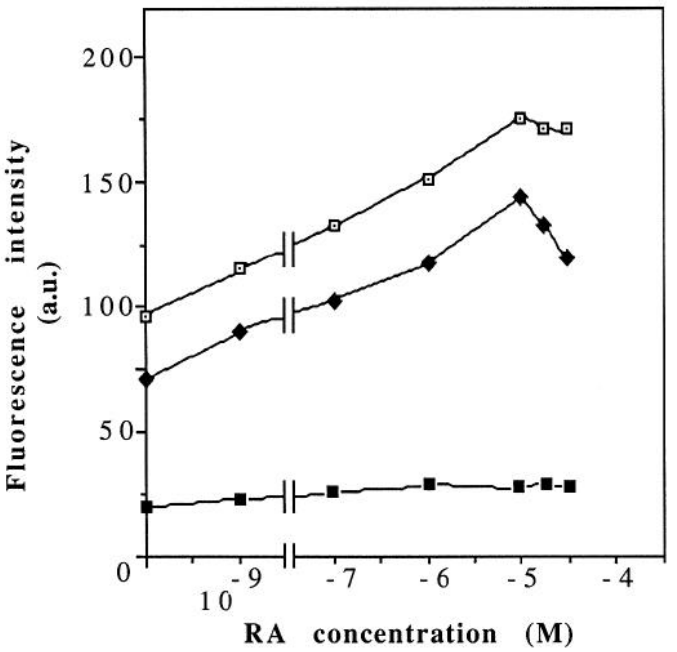

FIG. 3. Dose-dependent effect of RA on ICAM-1 expression in thyroid cells. NPA (□), TPC-1 (◇), and WRO (■) cells were treated with increasing concentrations of RA for 24 h. Cells were harvested, incubated with anti-ICAM-1 mAb, and analyzed by immunofluorescence flow cytometry. Representative results of one of three similar experiments are presented.

expression persisted for 3 days and returned to basal levels after 4 days.

Northern blot analysis of ICAM-1 in RA-treated thyroid cell lines

To analyze the mechanism underlying ICAM-1 surface enhancement, we performed Northern blot analysis in the RA-sensitive NPA and the RA-insensitive WRO cell lines.

In the NPA cell line, RNA blot analysis showed two transcript bands of 3.3 and 2.4 kilobases. In the same cell line, we found an increase in ICAM-1 mRNA after RA treatment (Fig. 4). Specific mRNA accumulation was more consistent at 72 h (2.5 ± 0.1-fold) than at 24 h (mean ± SE of three different experiments, 1.8 ± 0.12-fold). The WRO cell line, probably because of its low basal expression of ICAM-1, did not show any detectable band of ICAM-1 mRNA under basal condition or at different times of RA treatment.

Dependence of ICAM-1 expression on RNA and protein synthesis

NPA cells were treated with RA in the presence of 400 nmol/L (0.5 μg/mL) actinomycin-D or 106 μmol/L (30 μg/mL) cycloheximide. The concentrations of inhibitors were determined by preliminary experiments to establish the maximal nontoxic dose for each agent. After 24 h, cell cultures were analyzed by immunofluorescence flow cytometry. Actinomycin-D treatment was able to completely inhibit RA-induced ICAM-1 up-regulation when added simultaneously with RA. Similar results were obtained with cycloheximide treatment (Fig. 5).

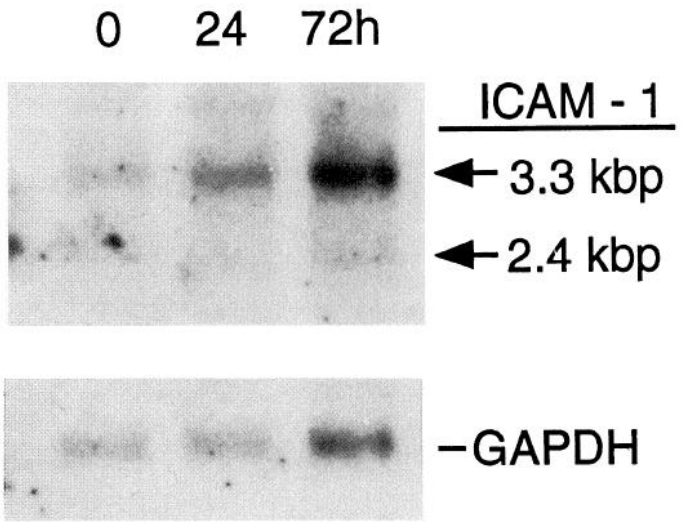

FIG. 4. Time-dependent modulation of ICAM-1 mRNA level by RA (10^{-5} mol/L) in the NPA cell line. Untreated or RA-treated cells were cultured up to 72 h. At the indicated time, untreated or RA-treated cells were harvested and subjected to Northern blot analysis, as described in *Materials and Methods*. The blots were analyzed by autoradiography. Autoradiograms were scanned by densitometer, and the ICAM-1 mRNA was normalized to the levels of GAPDH mRNA. The positions of ICAM-1 and GAPDH transcript bands are indicated.

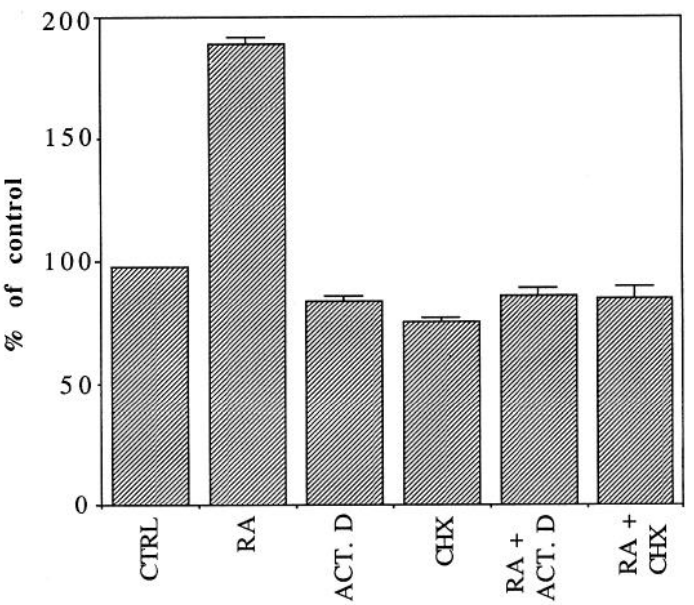

FIG. 5. The increased expression of ICAM-1 induced by RA depends on RNA and protein synthesis. Thyroid cells were incubated with or without RA (10^{-5} mol/L). Actinomycin-D (400 nmol/L; 0.5 μg/mL; ACT.D) or cycloheximide (106 μmol/L; 30 μg/mL; CHX) were added at time zero to control or RA-treated cells. At 24 h, cells were harvested, incubated with anti-ICAM-1 mAb, and analyzed by immunofluorescence flow cytometry. The MFI of ICAM-1 expression in untreated cells was considered 100%. Each *bar* represents the mean percentage ± SE of three independent experiments.

Modulation of ICAM-1 expression on thyroid cell lines by cytokines and RA

Cells were incubated for 24 h with 10 μmol/L RA and/or 200 U/mL of each cytokine. ICAM-1 surface expression, analyzed by immunofluorescence flow cytometry, showed a

higher increase when NPA cells were incubated with RA plus IFNγ or TNFα than when they were treated with RA or each cytokine separately (Fig. 6). Similar results were obtained with TPC-1 and ARO cell lines. In the WRO cell line, IFNγ induced only a small increase in ICAM-1 expression (1.7-fold), whereas TNFα did not significantly change its expression. Treatment with IFNγ or TNFα (200 U/mL) for up to 5 days or higher IFNγ or TNFα concentrations (1000 U/mL) for 24 h did not further increase ICAM-1 expression.

Northern blot analysis (Fig. 7) showed an increase, compared to control cell lines, in ICAM-1 mRNA accumulation when the RA-sensitive NPA cell line was incubated for 24 h with 10 μmol/L RA (1.8 ± 0.13-fold), IFNγ (4.0 ± 0.14-fold), or TNFα (2.5 ± 0.07-fold). A further increase in ICAM-1 accumulation was observed in cells incubated with 10 μmol/L RA plus IFNγ (7.2 ± 0.22-fold) or TNFα (mean ± SE of three different experiments, 4.8 ± 0.18-fold).

Cell cycle analysis of RA-treated thyroid cell lines

After 72 h of RA treatment, no significant change was found in cell number or cell distribution in cell cycle phases compared to control cultures.

Discussion

RA-induced ICAM-1 expression has been investigated in only a few cell lines with conflicting results (16–18). In the present study we investigated for the first time the effect of RA on ICAM-1 expression in human thyroid cell lines. Our results indicate that RA induced ICAM-1 up-regulation on the cell surface of human thyroid cells, in agreement with the findings of Wang *et al.* in melanoma cell lines (17) and Bouillon *et al.* (16) in one teratocarcinoma, one glioma, one melanoma, and one neuroblastoma cell line. In our system, ICAM-1 enhancement by RA was, generally, dose related, time dependent, and reversible. Furthermore, RA increased only the expression of ICAM-1, whereas no effect was observed on the expression of other CAMs, such as NCAM/CD56, an antigen present on thyroid cells (19, 20); the α_4-chain of the β_1-family of integrins, which are normally absent on normal thyroid cells (21) but expressed in thyroid tumors *in vivo* and *in vitro* (21a); or LFA-3, a molecule involved in the nonantigen-specific adhesion system. The observation that, independent of the effect of RA, thyroid tumor cells express ICAM-1 and LFA-3 molecules suggests that both nonantigen-specific adhesion pathways, LFA-1/ICAM-1 and CD2/LFA-3, are potentially able to mediate interactions with effector cells of the immune system.

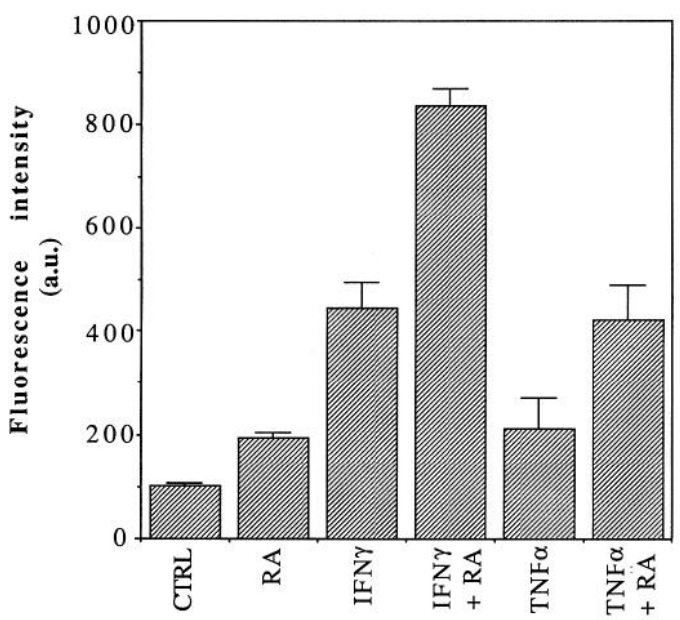

FIG. 6. Effect of RA on the increased expression of surface ICAM-1 induced by IFNγ or TNFα in NPA cells. Cells were treated for 24 h with RA (10^{-5} mol/L), IFNγ (200 U/mL), or TNFα (200 U/mL). Cells were harvested, incubated with anti-ICAM-1 mAb, and analyzed by immunofluorescence flow cytometry. Each *bar* represents the mean MFI ± SE of three independent experiments.

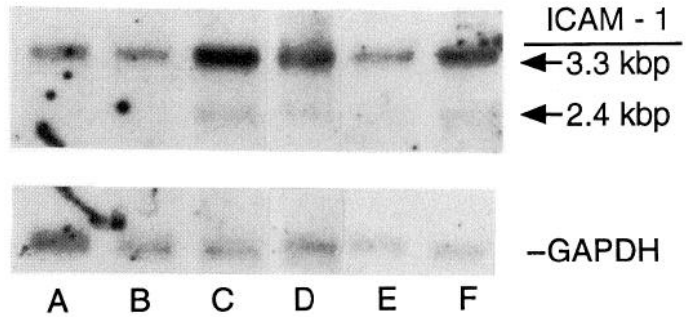

FIG. 7. Effect of RA on the increase in mRNA levels induced by IFNγ or TNFα in NPA cells. Untreated cells (A) or cells incubated with RA (B; 10^{-5} mol/L) IFNγ (200 U/mL; D), or TNFα (200 U/mL; E), alone or with RA plus IFNγ (C) or RA plus TNFα (F) were cultured for 24 h. Northern blot was performed as described in *Materials and Methods*. The blots were analyzed by autoradiography. Autoradiograms were scanned by densitometer, and the ICAM-1 mRNA was normalized to the level of GAPDH mRNA. The positions of ICAM-1 and GAPDH transcript bands are indicated.

On the other hand, RA treatment did not modify ICAM-1 expression in the WRO cell line, and not all the cells of TAD-2 line were RA responsive, suggesting that in the latter line, two or more cellular clones may coexist. This different RA susceptibility of the ICAM-1 response was also observed in melanoma cell lines by Wang *et al.* (17), who suggested that a variable expression of α, β, and γ RA receptors could be the cause of the different ICAM-1 response to RA treatment. Studies of RAR expression in our cell lines could be useful to test this hypothesis.

Cytokines, such as IFNγ and TNFα, both individually and to a greater extent in combination with RA, increased ICAM-1 expression in NPA, TPC-1, and ARO cell lines. These findings suggest that it is likely that these different agents used a different mechanism of ICAM-1 molecule induction. In contrast, the RA-insensitive WRO cell line showed a weak or absent ICAM-1 modulation with IFNγ or TNFα treatment, respectively. The low basal expression of ICAM-1 and LFA-3, antigens not expressed in all cells, and the weak or absent response to a wide number of ICAM-1-stimulating agents could be another aspect of the malignant behavior of this cell line in addition to the high tumorigenicity in nude mice (22)

and the lack of sensibility to interleukin-1 action on cell growth (23).

To understand the mechanism underlying ICAM-1 up-regulation, we investigated this phenomenon at the transcriptional level. A parallel increase in ICAM-1 mRNA was detected in RA-treated cell lines. This specific mRNA accumulation was dependent on mRNA synthesis, as demonstrated by the inhibition of ICAM-1 modulation after actinomycin-D treatment. Furthermore, simultaneous cycloheximide treatment showed that RA-induced ICAM-1 expression was totally dependent on protein neosynthesis. However, these data are not sufficient to conclude that the action of RA proceeds via specific responsive elements on the ICAM-1 gene. ICAM-1 modulation, in RA-stimulated cell lines, could be due to *de novo* production of a RA-induced growth factor(s) or cytokine(s). Indeed, in other cellular systems, such as cultured keratinocytes and epidermis *in vivo*, RA-induced inhibition of DNA synthesis is mediated by transforming growth factor-β2, a growth factor produced *de novo* (24). For the cell lines we used, NPA cells under basal conditions are able to produce a novel platelet-derived growth factor-like protein, whereas WRO cells express fibroblast growth factor (25). However, spent medium of 72-h RA-treated NPA or TPC-1 cells was unable to induce ICAM-1 stimulation in the RA-insensitive WRO cell line (our unpublished data). Furthermore, in contrast with the glioma cell line (26), protein kinase-A stimulators, such as forskolin and 8-bromo-cAMP, had no effect on ICAM-1 expression in thyroid cell lines. On the other hand, a protein kinase-C stimulator, 12-*O*-tetradecanoylphorbol-13-acetate induced, in some, but not all, RA-sensitive thyroid cell lines, an earlier and stronger increase in ICAM-1 expression compared to the slower RA-induced modulation (our unpublished observation). Therefore, a hypothetical RA-induced growth factor(s)/cytokine(s) or transduction pathway, such as protein kinase-A or -C, is unlikely to be involved in ICAM-1 modulation by RA in thyroid cell lines. These observations, supported by the known presence of RA-specific responsive elements in the 5′-regulatory region up-stream of the human ICAM-1 gene (27), suggest that the ICAM-1 gene in our system probably represents a gene that is transcriptionally regulated by RA at the level of specific responsive elements.

Conflicting results have been reported on the effect of RA on HLA antigens (17, 28). We did not detect substantial changes in the expression of HLA class I and DR, with the exception of the ARO and NPA cell lines, respectively. These findings suggest that RA-induced effects on HLA antigens are not a general mechanism, but could be related to a different susceptibility of each cell line.

In conclusion, an understanding of the mechanisms underlying the modulation of an important molecule, such as ICAM-1, may give a new insight into the biology of thyroid tumors and their possible interactions with the immune system. In particular, increased levels of ICAM-1 expression may be important in the control of growth and persistence of tumoral cells by immunological mechanisms. *In vitro* assays of cell adhesion with lymphocytes or cytotoxicity with ^{51}Cr may help to understand the role of ICAM-1 modulation and the potential use of RA and proinflammatory cytokines in control of tumor growth. Moreover, the wide spectrum of susceptibility to RA in thyroid cell lines may be a useful model to investigate the mechanism of RA action in gene regulation and the correlation between RA-induced effects and expression of RA receptors.

Acknowledgments

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