Differential Susceptibility of Cultured Human Melanoma Cell Lines to Enhancement by Retinoic Acid of Intercellular Adhesion Molecule 1 Expression

Zhigang Wang, Yan Cao, Claudio M. D'Urso, and Soldano Ferrone

Department of Microbiology and Immunology, New York Medical College, Valhalla, New York 10595

ABSTRACT

The potential role of intercellular adhesion molecule 1 (ICAM-1) in the biology of human melanoma cells has stimulated interest in the characterization of its modulation. The present study has shown that the differentiating agent retinoic acid (RA) up-regulates ICAM-1 expression by melanoma cells in a dose- and time-dependent fashion. The enhancement of ICAM-1 cell surface expression is paralleled by an increase in ICAM-1 mRNA. Therefore, ICAM-1 represents an additional gene which may be transcriptionally regulated by RA. The five melanoma cell lines tested displayed a differential susceptibility to the modulation of ICAM-1 expression by RA, since the cell line MeWo did not change in its ICAM-1 expression following incubation with RA. Nevertheless, RA-insensitive as well as RA-sensitive melanoma cell lines displayed a higher increase in ICAM-1 expression following incubation with RA and cytokines than following incubation with each of them. Analysis of the distribution in the melanoma cell lines of retinoic acid receptors (RARs) showed a relationship between susceptibility to RA-mediated increase of ICAM-1 expression and RARβ expression, suggesting that the latter receptor may play a role in the phenomenon. RARα and RARγ were present in RA-sensitive and -insensitive melanoma cell lines, suggesting that they play a role in the enhancement by RA of cytokine-mediated up-regulation of ICAM-1 expression. The melanoma cell lines we have described may represent a useful system for investigating the role of RAR in the regulation of gene expression and the mechanism(s) underlying this effect.

INTRODUCTION

The malignant transformation of human melanocytes may be associated with changes in their antigenic profile (for a review, see Refs. 1 and 2). Whether these changes reflect a differentiation-related phenomenon or represent an epiphenomenon of the malignant transformation of melanocytes remains to be determined. We have approached this question by correlating the effect of differentiating agents on the expression of cell surface markers and on the differentiation of melanoma cells. In the course of these investigations, we have observed that cultured human melanoma cells display a differential susceptibility to the enhancement by the differentiating agent retinoic acid RA1 of ICAM-1 expression. The latter molecule may play a role in the interaction of melanoma cells with immune cells (3, 4), in the metastatic process, and in the clinical course of the disease (5, 6). In the present investigation, we have characterized the enhancement by RA of ICAM-1 expression by cultured melanoma cells and the mechanism(s) underlying this phenomenon, since this information may contribute to our understanding of the regulation of ICAM-1 expression by melanoma cells.

MATERIALS AND METHODS

Cell Lines. The melanoma cell line A375 was cultured in a mixture of Dulbecco's minimal essential medium (Gibco laboratories, Grand Island, NY) and 50% Nutrient mixture F-12 (Ham) (Gibco) supplemented with 8% fetal calf serum and 2 mm L-glutamine. The melanoma cell lines Colo 38, MeM50–10, MeWo, 3S5, and 70W were grown in opti-minimal essential medium (Gibco) supplemented with 5% fetal calf serum and 2 mm L-glutamine.

mAb. The anti-HLA Class I mAb W6/32, the anti-HLA-DR, DQ, DP mAb Q5/13, the anti-ICAM-1 mAb CL207.14, and the anti-HMW-MAA mAb 763.74 were prepared and characterized as described elsewhere (7–11).

mAb were purified from ascitic fluid by sequential precipitation with caprylic acid and ammonium sulfate (12). mAb were radiolabeled with 125I utilizing the Iodo-gen method (13). The immunoreactive fraction of radiolabeled mAb was at least 50%, as determined by the method of Lindmo et al. (14).

Chemicals and Cytokines. RA was purchased from Aldrich Chemical Inc. (Milwaukee, WI). IFN-γ, IL-1, and TNF-α were obtained from Hoffman-LaRoche Inc. (Nutley, NJ), from Immunex Corporation (Seattle, WA), and from Cetus Corporation (Emeryville, CA), respectively. cDNA Probes. The ICAM-1 cDNA probe pG4H1.1 MS-4 (15), the ICAM-2 cDNA probe pg7/E, B2 (16), the hRARα, β, and γ cDNA probes (17, 18), the α-actin cDNA probe (19), and the β-actin cDNA probe (20) were prepared as described. Three-kilobase ICAM-1 cDNA probe was isolated by digestion of plasmid with Sal and EcoRI; 0.6-kilobase hRARα cDNA probe by digestion with PstI; 0.7-kilobase ICAM-2, 0.6-kilobase hRARβ, and 1.6-kilobase hRARγ cDNA probes by digestion with EcoRI; 0.8-kilobase α-actin cDNA probe by digestion with BamHI and PstI; and 0.7-kilobase β-actin cDNA probe by digestion with BamHI and EcoRI. cDNA fragments were then electrophoresed, excised from low-melting-point agarose gel and radiolabeled with [α-32P]dCTP by random priming (21) at the specific activity of 1 x 108 cpm/μg.

Binding Assays. Binding of 125I-labeled mAb to cells was measured in 96-well V-bottomed microtiter plates (Becton Dickinson and Co., Oxnard, CA) as described (10). Briefly, cells (1 x 105) were mixed with 125I-labeled mAb (1 x 106 cpm) in a total volume of 100 μl of phosphate-buffered saline containing 1% bovine serum albumin. Following a 90-min incubation at 4°C on a rotator, cells were pelleted by centrifugation at 2000 rpm for 5 min. Supernatant was then removed, and cell-bound radioactivity was measured in a γ counter (LKB-1261 LKB-Wallac, Turku, Finland). Nonspecific binding was determined by incubating 125I-labeled mAb with cells in the presence of excess nonradiolabeled mAb. Results are expressed as specifically bound cpm/1 x 105 cells. Binding of 32P-labeled mAb in cells was measured as described elsewhere (22–24).

Radiolabeling of Cells, Immunoprecipitation, and SDS-PAGE. These assays were performed as described elsewhere (10). Briefly, labeling with 125I using the lactoperoxidase method (25), cells were solubilized by incubation for 30 min at 4°C in lysis buffer containing 1% Nonidet P-40, 10 mM Tris-HCl (pH 8.2), 0.15 mM NaCl, 1 mM EDTA, 1 mg/ml bovine serum albumin, and 1 mM phenylmethylsulfonyl fluoride. The cell extract was then incubated for 12 h at 4°C with AFFI-GEI 10 (Bio-Rad Laboratories, Richmond, CA) coupled with mAb CL207.14. One-dimensional SDS-PAGE analysis was performed.
under reducing conditions in slab gels containing 3–15% polyacrylamide and utilizing the buffer system described by Laemmli (26). Gels were processed for autoradiography using Kodak XAR-5 film (Eastman Kodak Company, Rochester, NY).

Northern Blot Analysis. Total RNA extraction, electrophoresis, blotting, hybridization, and autoradiography were performed as described elsewhere (27). Blots were reused after the probes had been removed by incubation in a solution containing 0.1x SSC and 0.1% SDS at 98°C for 5 min. The magnitude of hybridization was quantitated by scanning densitometry using a GS-300 transmittance/reflectance scanning densitometer (Heuffer Scientific Instruments, San Francisco, CA).

RESULTS

Following a 3-day incubation at 37°C with RA (10^{-6} M) cultured melanoma cells MeWo did not change in their reactivity with 125I-anti-ICAM-1 mAb CL207.14, while cultured melanoma cells A375, Colo 38, MeM50–10, 3S5, and 70W displayed an increased reactivity. The extent of enhancement was about 3-fold for Colo 38 cells and about 2-fold for the other cell lines. The reactivity with 125I-mAb CL207.14 of all the cell lines did not change following a 3-day incubation at 37°C in medium supplemented with 0.01% ethanol. The latter is used to dissolve RA. RA induced no change in the reactivity of all the cell lines with 125I-anti-HLA Class I mAb W6/32, 125I-anti-HLA Class II mAb Q5/13, and 125I-anti-HMW-MAA mAb 763.74, except for a slight decrease in the reactivity with the latter mAb of Colo 38 and 70W cells (Table 1). Furthermore, RA did not induce the expression of ICAM-2 by the melanoma cell lines tested, as measured by Northern blotting analysis with the ICAM-2 cDNA probe pG7/E,B.2 (data not shown). Serological assays could not be utilized to analyze the cell surface expression of ICAM-2, since anti-ICAM-2 antibodies are not available to us.

Additional experiments were performed to characterize the variables and the mechanisms of the modulation by RA of ICAM-1 expression. Colo 38 cells, which are the most sensitive to RA, and MeWo cells, which are not sensitive to modulation by RA of ICAM-1 expression, were used in these experiments. The effect of RA on the expression of ICAM-1 by Colo 38 cells is dose dependent, the maximal increase being obtained at the concentration of 10^{-5} M (Fig. 1A). At all the concentrations of RA tested ranging between 10^{-7} and 10^{-5} M, no effect was detected on the expression of ICAM-1 by MeWo cells. The enhancement by RA of ICAM-1 expression by Colo 38 cells is time dependent. Following a 12-h incubation with RA (10^{-5} M), ICAM-1 expression was markedly increased and reached a plateau following a 24-h incubation (Fig. 1B). After a 24-h incubation with RA, HMW-MAA expression was decreased by about 27%. No change in HLA Class I and Class II antigen expression was detected. The up-regulating effect on ICAM-1 expression by RA is reversible. When Colo 38 cells were cultured in RA-free medium following a 24-h incubation with RA, ICAM-1 expression remained high during the first 2 days of culture, decreased by about 50% following 4 days of incubation, and returned to basal levels following 7 days of incubation (data not shown).

Table 1 Enhancement by retinoic acid of ICAM-1 expression by cultured human melanoma cells

<table>
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<tr>
<th>Cell line</th>
<th>RA (10^{-6} M)</th>
<th>Anti-ICAM-1 mAb CL207.14</th>
<th>Anti-HLA Class I mAb W6/32</th>
<th>Anti-HLA Class II mAb Q5/13</th>
<th>Anti-HMW-MAA mAb 763.74</th>
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<tr>
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<td>101.1</td>
<td>34.6</td>
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<td>108.8</td>
<td>175.7</td>
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</table>

* Melanoma cells were incubated with retinoic acid (10^{-6} M) for 72 h at 37°C. Then cells were washed and tested with 125I-labeled mAb in a binding assay.

* +, with retinoic acid; -, without retinoic acid.

cpm × 10^{-3}.

Fig. 1. Dose- and time-dependent enhancement by RA of ICAM-1 expression by cultured melanoma cells Colo 38 and MeWo. Colo 38 (——) and MeWo (⋯⋯⋯⋯) cells were incubated at 37°C with up to 10^{-5} M of RA for 24 h (A) or with RA (10^{-5} M) for up to 72 h (B). Cells were then harvested and tested with 125I-labeled anti-ICAM-1 mAb CL207.14 (○), anti-HLA Class I mAb W6/32 (■), anti-HLA Class II mAb Q5/13 (□), and anti-HMW-MAA mAb 763.74 (♦) in a binding assay. The experiment was repeated three times with reproducible results. Representative results are presented. Points, mean of triplicates. SD was less than 10%.
In order to define the molecular basis of the increased serological reactivity with anti-ICAM-1 mAb of RA-treated melanoma cells, antigens immunoprecipitated by anti-ICAM-1 mAb CL207.14 from RA-treated and control Colo 38 and MeWo cells were analyzed by SDS-PAGE. The intensity of the Mr, 96,000 component immunoprecipitated from RA-treated Colo 38 cells was stronger than that from untreated cells (Fig. 2). It is noteworthy that no difference was detected in the intensity of the Mr, 96,000 component immunoprecipitated by mAb CL207.14 from RA-treated and control MeWo cells.

To analyze the mechanism(s) underlying the differential modulation of ICAM-1 on Colo 38 and MeWo cells by RA, the steady state level of mRNA for ICAM-1 was analyzed by Northern blot hybridization in the two cell lines following incubation with RA for up to 72 h (Fig. 3). Both cell lines contain RNA molecules which hybridize to ICAM-1 cDNA probe pG4H1. 1MS-4. Only in Colo 38 cells did RA enhance the level of ICAM-1 mRNA by about 3.5-fold after a 12-h incubation and by about 5-fold after a 24-h incubation. No further increase was found after a 48-h incubation with RA. The effect is specific, since no difference between RA-treated and control Colo 38 cells was detected in the level of RNA hybridizing with β-actin cDNA probe.

Additional experiments measured the level of hRARα, hRARβ, and hRARγ mRNA in Colo 38 and MeWo cells. Two species of hRARα mRNA at sizes of 3.8 and 2.8 kilobases were detected in both cell lines (Fig. 4). The level of mRNA was similar in the two cell lines. The 2.8-kilobase mRNA species was slightly increased in Colo 38 and MeWo cells incubated with 10^-5 M RA for 8 and 48 h, respectively. Two species of hRARβ mRNA with at sizes of 3.6 and 3.1 kilobases were detected only in Colo 38 cells (Fig. 4). After treatment with 10^-5 M RA for up to 72 h hRARβ mRNA was still not detected in MeWo cells; its level in Colo 38 cells was increased by about 4-fold following a 4-h incubation with 10^-5 M RA and by about 10-fold following a 12-h incubation. No additional increase was observed by prolonging the incubation up to 48 h. A slight decrease was observed following a 72-h incubation. RARγ mRNA (3.2 kilobases) was detected in both Colo 38 and MeWo cells (Fig. 4). Its level increased slightly in Colo 38 cells after a 12-h incubation with RA and reached an ~2.5-fold increase after a 72-h incubation. No change in the level of RARγ mRNA was detected in MeWo cells incubated with RA for up to 72 h.

RA has been shown to enhance the antiproliferative and differentiating effect of IFN-γ and TNF-α on leukemic (24, 28) and neuroblastoma (29) cell lines. These cytokines and IL-1 have been shown to up-regulate ICAM-1 expression by melanoma cells (10). Therefore, the effect of the combination of RA with IFN-γ, IL-1, or TNF-α on ICAM-1 expression by melanoma cells was investigated. The combination of RA with IFN-γ or TNF-α enhanced ICAM-1 expression by Colo 38 cells more than the individual agents did separately; the increase induced by the combination of RA plus IFN-γ was greater than that induced by the combination of RA plus TNF-α (Fig. 5). The increase induced by the combination of RA and IL-1 was similar to that induced by RA alone. RA enhanced the increase of ICAM-1 expression induced by the three cytokines on MeWo cells; the combination of RA and TNF-α had the largest effect, and the combination of RA and IL-1 had the smallest one. The level of ICAM-1 expression induced by RA in combination with the cytokines on Colo 38 cells was higher than that induced on MeWo cells, although each of the three cytokines enhanced by itself the level of ICAM-1 expression on the
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**Fig. 4.** Time-dependent modulation by RA of RAR mRNA level in cultured melanoma cells Colo 38 and MeWo. Colo 38 and MeWo cells were incubated at 37°C with RA (10^-5 M) for up to 72 h. At the indicated times, cells were harvested and washed. Total cytoplasmic RNA was then extracted. Total RNA (20 μg/lane) was size fractionated in a 1% agarose-formaldehyde gel and blotted onto a nitrocellulose membrane. The blot was sequentially hybridized with 32P-labeled hRARα, hRARβ, and hRARγ cDNA probe. Before rehybridization, each probe was eluted by incubating the blot for 5 min at 98°C in 0.1 x SSC solution. After each hybridization, the blot was analyzed by autoradiography (A). Autoradiograms were densitometrically scanned. The values for the levels of RARα (O), RARβ (△), and RARγ (□) mRNA were normalized to that of actin mRNA and plotted versus time after plating (B).

DISCUSSION

It has been known for some time that ICAM-1 expression in various types of cells is enhanced by cytokines (10). This study extends to RA the ability to up-regulate ICAM-1 expression on human melanoma cell lines. The modulation of ICAM-1 expression by RA shares some characteristics with cytokines but also displays some unique properties. Like that of cytokines, the effect of RA is time and dose dependent, is reversible, is associated with an increase of ICAM-1 mRNA level, and is not mediated by an effect on cell differentiation. At variance with cytokines which modulate ICAM-1 expression on all the melanoma cell lines tested, although to a different extent (10), RA enhanced ICAM-1 expression on most but not all of the melanoma cell lines tested. An additional difference with IFN-γ and TNF-α is represented by the lack of marked effects of RA on the expression of HLA Class I and Class II antigens and HLA Class I antigen expression by Colo 38 and MeWo cells. RA slightly decreased the enhancement by TNF-α of HLA Class I antigen expression by Colo 38 cells.

To determine whether the enhancing effect of RA on the modulation of ICAM-1 expression by cytokines was caused by changes in the number and/or affinity of IFN-γ and TNF-α receptors expressed by Colo 38 and MeWo cells, these parameters were analyzed. Scatchard plot analysis of the binding of 32P-IFN-γ and 125I-TNF-α to the two cell lines detected no significant change in the number of receptors for IFN-γ and TNF-α or in their affinities following a 24-h incubation with 10^-5 M RA (data not shown).

To determine whether the modulation of ICAM-1 expression by RA on melanoma cells was associated with an effect on their differentiation and proliferation, a culture of each cell line was incubated with RA (10^-5 M) for up to 30 days. A culture incubated in medium without RA was used as a control. At weekly intervals cells were examined microscopically. No change in their morphology was observed. Furthermore, the cell concentration in the culture incubated with RA for 6 days was not different from that in the control culture. However, following a 2-week incubation with RA, the cell concentration was about 30% lower than in the control culture.

Two cell lines were treated with RA in combination with each of the three cytokines (Fig. 6). It is noteworthy that mRNA for ICAM-2 was not detected in the melanoma cell line Colo 38 following incubation with RA in combination with IFN-γ, IL-1, or TNF-α (data not shown). In addition, RA had no effect on the modulation by IFN-γ of HLA Class I and Class II antigen expression by Colo 38 and MeWo cells. RA slightly decreased the enhancement by TNF-α of HLA Class I antigen expression by Colo 38 cells.

To determine whether the enhancing effect of RA on the modulation of ICAM-1 expression by cytokines was caused by changes in the number and/or affinity of IFN-γ and TNF-α receptors expressed by Colo 38 and MeWo cells, these parameters were analyzed. Scatchard plot analysis of the binding of 32P-IFN-γ and 125I-TNF-α to the two cell lines detected no significant change in the number of receptors for IFN-γ and TNF-α or in their affinities following a 24-h incubation with 10^-5 M RA (data not shown).

To determine whether the modulation of ICAM-1 expression by RA on melanoma cells was associated with an effect on their differentiation and proliferation, a culture of each cell line was incubated with RA (10^-5 M) for up to 30 days. A culture incubated in medium without RA was used as a control. At weekly intervals cells were examined microscopically. No change in their morphology was observed. Furthermore, the cell concentration in the culture incubated with RA for 6 days was not different from that in the control culture. However, following a 2-week incubation with RA, the cell concentration was about 30% lower than in the control culture.

The modulation of ICAM-1 expression by RA has been investigated in only a few human cell lines. The results have been conflicting. RA was found to reduce ICAM-1 expression and to induce a more differentiated phenotype in two doxorubicin variants of the human colon adenocarcinoma cell line LoVo (31). If not caused by technical artifacts, the discrepancy between these
The experiment was repeated three times with reproducible results. Representative results are presented. Points, mean of triplicates. SD was less than 10%.

results and our own may reflect differences in the regulatory mechanisms controlling ICAM-1 expression in cells of different lineage. An alternative, although not exclusive, possibility is represented by the fact that the reduction in ICAM-1 expression by LoVo cells is not a direct effect of RA but reflects an antigenic change associated with the induction of cell differentiation by RA. In contrast, ICAM-1 expression was found to be increased by RA on one glioma, one melanoma, one neuroblastoma, and one teratocarcinoma cell line (32, 33). In agreement with our results, the enhancement of ICAM-1 expression by RA was associated with an increase in ICAM-1 mRNA, was reversible, and was time dependent (32), although the kinetics was different from that in melanoma cell lines. Modulation of ICAM-1 expression by RA in the glioma cell line tested appears to be mediated by the cAMP-dependent transduction pathway (33). Whether the latter pathway is also involved in the enhancement of ICAM-1 expression by RA on melanoma cells Colo 38 is not known, since the lack of effect of the protein kinase A inhibitor HA1004 on the phenomenon* may reflect the activation of multiple pathways by RA.

The up-regulation of ICAM-1 cell surface expression by RA is paralleled by an increase in ICAM-1 mRNA. This finding in conjunction with the detection of the postulated retinoic acid-responsive element sequence in the first intron of the ICAM-1 gene (34) suggests that ICAM-1 represents another gene which is transcriptionally regulated by RA. Analysis of the kinetics of the increase by RA of the level of ICAM-1 mRNA showed that the time for its induction is intermediate between that required for "early" and that for "late" RA-induced genes. ICAM-1 mRNA increased following a 12-h incubation with RA, while early RA-induced genes like ERα (Hox-16) (35) and RARβ gene (36, 37) increase within the first 3 h of incubation with RA, and late RA-induced genes like laminin B1 (38) increase after 24 h of incubation with RA.

Fig. 5. Effect of RA on the enhancement by IFN-γ, IL-1, and TNF-α of ICAM-1 expression by cultured melanoma cells Colo 38 and MeWo. Colo 38 (A) and MeWo (B) cells were incubated at 37°C for 24 h with RA (10^-5 M), IFN-γ (1000 units/ml), IL-1 (1000 units/ml), and/or TNF-α (1000 units/ml). Cells were then harvested and tested with 125I-anti-ICAM-1 mAb CL207.14, 125I-anti-HLA Class I mAb W6/32, and 125I-anti-HLA Class II mAb Q5/13 in a binding assay. The experiment was repeated three times with reproducible results. Representative results are presented. Points, mean of triplicates. SD was less than 10%.

One interesting result of our studies has been the differential susceptibility of the various melanoma cell lines tested to modulation of ICAM-1 expression by RA. The lack of susceptibility to modulation of MeWo cells does not reflect a general resistance to RA, since the latter enhanced the antigenic modulation induced by cytokines in MeWo cells. It is intriguing that while RARα and RARγ were expressed in all the cell lines tested, RARβ was expressed in the RA-sensitive cell line but was not detectable and not inducible by RA in the RA-resistant cell line. If this association does not represent a fortuitous event, our results suggest that the RA-mediated regulation of ICAM-1 gene expression may be dependent on RARβ expression and that the differential expression of RARβ accounts for the differential susceptibility to RA-mediated enhancement of ICAM-1 expression. This possibility is supported by the temporal relationship between ICAM-1 mRNA up-regulation and RARβ gene induction by RA, since the latter has been suggested to be itself inducible by RA and to play a role in triggering a number of relatively late RA-mediated responses (36, 37). We found that RARβ transcripts were induced by RA several hours before ICAM-1 transcripts were increased. Our results do not rule out a role for RARα and RARγ in the modulation by RA of ICAM-1 expression, since RA enhanced the cytokine-mediated up-regulation of ICAM-1 expression by MeWo cells, which express RARα and RARγ but lack RARβ. It is of interest that this effect of RA is not associated with marked changes in the amount of IFN-γ and TNF-α bound by melanoma cells or in the binding affinity of the corresponding receptors. Therefore, the effect of RA appears to occur at a postmembrane level, independent of cytokine-receptor interactions. If so, this mechanism resembles that proposed to underlie the synergistic effect of RA and IFN-γ on the differentiation and growth inhibition of neuroblastoma cells (29) but not of RA and TNF-α on the growth inhibition of normal hematopoietic and leukemic cells (24).

Differential expression of the various types of RAR is not unique to MeWo cells, since RARβ mRNA was not detected in some human hematopoietic (36), liver carcinoma (18), and squamous cell carcinoma (39, 40) cell lines; in human keratinizing epithelial cells (40); or in some murine melanoma cells (41), all of which express RARα and/or RARγ. Furthermore, the distribution of RARβ in the developing limbs of mice is different from that of RARα and RARγ (42). Two additional characteristics of the tissue distribution of the various types of RAR are of interest in view of our results in MeWo cells. In human hematopoietic cell lines suppression of RARβ gene expression is associated with an overexpression of RARα mRNA (36). This association was not found in MeWo cells, since the level of RARα mRNA was similar to that in Colo 38 cells which express RARβ mRNA. Furthermore, MeWo cells resemble cultured human squamous cell carcinoma cells and some mouse melanoma cell lines in their lack of susceptibility of RARβ to induction by RA (39–41). All of them differ from human hepatoma cell lines, mouse teratocarcinoma cells, and some
Fig. 6. Effect of RA on the enhancement by IFN-γ, IL-1, and TNF-α of ICAM-1 mRNA level in cultured melanoma cells Colo 38 and MeWo. Colo 38 and MeWo cells were incubated at 37°C for 24 h with RA (10⁻⁵ M), IFN-γ (1000 units/ml), IL-1 (1000 units/ml), and/or TNF-α (1000 units/ml). Cells were then harvested and washed twice. Total cytoplasmic RNA was extracted. Total RNA (15 μg/lane) was size fractionated in a 1% agarose-formaldehyde gel and blotted onto a nitrocellulose membrane. The blot was hybridized with 32P-labeled ICAM-1 cDNA probe and analyzed by autoradiography. The probe was then eluted by incubating the blot for 5 min at 98°C in 0.1x SSC solution. The blot was rehybridized with 32P-labeled α-actin cDNA probe and analyzed by autoradiography (A). Autoradiograms were densitometrically scanned. The values for the levels of ICAM-1 mRNA were normalized to that of actin mRNA (B).

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mouse melanoma cell lines, which up-regulate their levels of RARβ following incubation with RA (36).

Scanty and conflicting information is available about the effect of RA on HLA Class I and Class II antigen expression. de Vries et al. (43) reported an enhancement of HLA Class I and HLA-DR antigen expression by two human melanoma cell lines. On the other hand, in agreement with our results, Gross et al. (44) did not detect any change in the expression of HLA Class I and Class II antigens by neuroblastoma cell lines. Like the melanoma cell lines we analyzed, the neuroblastoma cell lines are susceptible to modulation by RA, since they displayed an increase in the expression of mAb Mel4/D12-defined MAA.

The selective effect of RA on ICAM-1 expression by melanoma cells suggests that this system will be a useful model for analyzing the regulatory mechanisms which control ICAM-1 expression and the role of ICAM-1 in the biology of melanoma cells and in their interaction with immune cells. Furthermore, the differential expression of the various types of RAR and the related differential susceptibility of melanoma cell lines to enhancement by RA of ICAM-1 expression provide a useful system for investigating the role of RAR in the regulation of gene expression and the mechanism(s) which underlie this effect.
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Differential Susceptibility of Cultured Human Melanoma Cell Lines to Enhancement by Retinoic Acid of Intercellular Adhesion Molecule 1 Expression

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