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Induction of Interferon by Transformed Cells: Inhibition by Retinoic Acid

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Retinoic acid (RA) inhibited transformed mouse L-929 and human WISH cell induction of interferon alpha/beta production by nonsensitized mouse spleen cells. The RA effect was both time and dose dependent and acted in near physiologic concentrations. The results suggest that the effect is due to a modulation of a previously described transformed cell surface associated glycoprotein IFN inducer.

Transformed cells as a class of interferon (IFN) inducer was first described by Svet-Moldavsky et al. (1) by the demonstration that injection of tumor cells into non-sensitized mice resulted in the production of IFN. Subsequently, it was shown that non-sensitized human lymphocytes produced IFN when cultured with virally and spontaneously transformed cells (2). The IFN produced was shown to be alpha/beta (α/β) in the mouse and αIFN in the human (3,4). The induction of the IFN by transformed cells has been shown to be caused by the interaction of a specific lymphocyte receptor with a glycoprotein inducer on the surface of the transformed cell (5,6). The inducer glycoproteins from several transformed cell types have been isolated and characterized and appear to be specific for the particular cell. Their role in

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transformed cell growth or tumor directed immune responses remain to be fully ascertained.

Retinoic (Vitamin A) acid (RA) has been shown to effect the expression of several cell surface molecules (7,8,9,10). In addition, RA can modulate the transformed cell phenotype by, for example, restoring density dependent contact inhibition of growth to transformed mouse L929 cells and anchorage dependent growth to L929, B16C3, and HeLa cells (11,12). Conceivably, the latter effects may be due to the alteration of the transformed cell surface. Since the IFN inducing molecules are on the cell surface and the cell surface is modified by RA, we carried out this study to determine the effect of RA on the interferon inducing potential of two transformed cell lines, mouse L-929 and human WISH cells, in non-sensitized mouse spleen cells.

MATERIALS AND METHODS

Mice. BALB/c mice were obtained from Texas Inbred Mouse Company, Houston, TX. Mice were used between 8 and 16 weeks of age.

Cells. Mouse L-929 and human WISH cells were obtained from the American Type Culture Collection. Cells were routinely grown to confluency in 32 oz. plastic culture flasks or microtiter plates in Eagles minimal essential medium with Earles salts supplemented with 10% fetal calf serum (FCS), penicillin (100 units), streptomycin (100 µg/ml) and garamycin (100 units) (EMEM 10%). Mouse spleen cell suspensions, inducer and IFN assays employed EMEM with 2% FCS.

Preparation of mouse spleen cell suspensions. Mice were euthanized with CO_2, their spleens asceptically removed and gently dissociated into single cell suspensions between glazed glass slides.

Assay of cell associated IFN inducing activity. L-929 or WISH cells were grown to confluency in the presence or absence of RA (all trans, Sigma Chemicals, St. Louis, MO) in EMEM 2% in microtiter plates for 24-48 hours. Prior to cocultivation with mouse splenocytes, L-929 or WISH cells were washed 3 times with EMEM 2% or phosphate buffered saline to remove RA. After washing, $1x10^6$ spleen cells in EMEM 2% were placed in each well and incubated for 24 hrs for IFN production.

Assay of soluble IFN inducing activity. L-929 or human WISH cells ($5x10^6$) were placed in 75 cm^2 plastic tissue culture flasks in the presence or absence of various concentrations of RA. Twenty-four to 48 hours after initiation of culture, cells were harvested from the flasks with a rubber policeman, washed 3 times with EMEM 2% and resuspended in 5 ml of the same medium. The cells were then sonicated at 40 KHz (Branson E-module) at room temperature for 1 minute and then centrifuged at 1000xg for 5 minutes. Varying dilutions of the supernatant fluid was employed as the inducer preparation. One-tenth of 1 ml of the inducer dilutions was then incubated with 0.1 ml spleen cells at $1x10^7$/ml for 24 hours for IFN production.

Interferon assays. Following incubation, duplicate samples were collected and assayed for IFNα/β activity on L-929 cells as previously described (13).

RESULTS

RA treatment of L-929 and WISH cells inhibits their ability to induce IFNα/β in mouse spleen cells. L-929 and human WISH cells were grown in the presence of 10^{-5}, $10^{-5.5}$, or 10^{-6} M RA or media for 48 hours prior to cocultivation with mouse spleen cells. If RA affected the IFN inducing potential of the L-929 or WISH cells, one would expect an RA dose dependent change in the amount of IFN produced by the mouse spleen cells. The extensive washing of the inducing cells would minimize the effects, if any, of residual RA remaining in the culture on the splenocytes. Figure 1 illustrates the results of such an experiment. The IFN inducing potential of L-929 cells appeared to be more sensitive to RA than did WISH cells, since a significant reduction in the IFN yield was demonstrated with as low as 10^{-6} M RA in the former

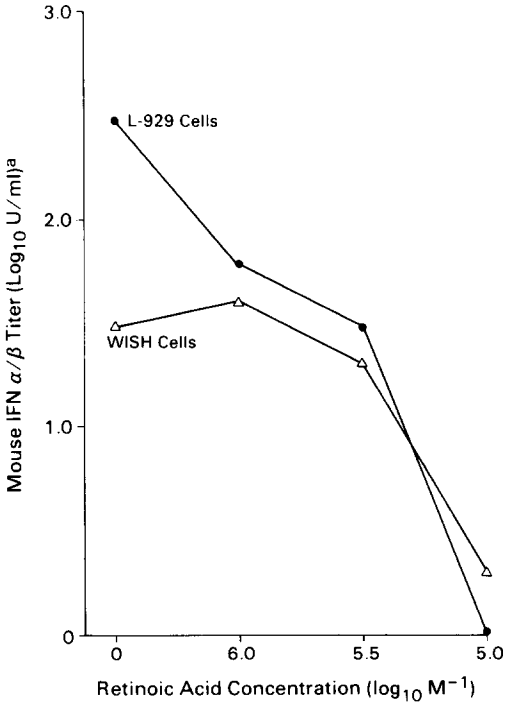

FIGURE 1: RA treatment of L-929 and WISH cells inhibits their ability to induce IFNα/β in mouse spleen cells.
L-929 and WISH cells were treated with the indicated concentrations of RA for 48 h prior to cocultivation with non-sensitized mouse spleen cells.
a) Differences in titer of IFNα/β $\geq$ 0.5 log are significant at P $\leq$ .05.

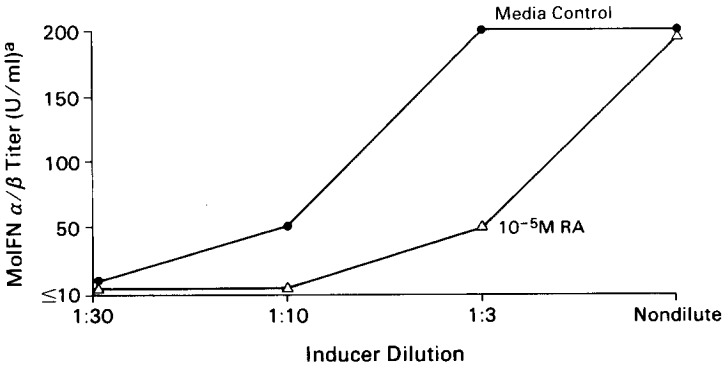

FIGURE 2: Effects of RA on the level of solubilized IFN inducing activity. Mouse L-929 cells were treated with 10^{-6} M RA for 48 h prior to production of soluble inducer (see Methods).
a) A 3-fold reduction of IFN titer is significant at $P \leq 0.05$.

and between $10^{-5.5}$ and $10^{-5.0}$ M RA in the latter. Higher concentrations of RA could not be tested at concentrations equal to or greater than $10^{-4.5}$ M since they were toxic to the inducing cell.

Effects of RA on the level of the solublized IFN inducing activity. We have shown that intact L-929 and WISH cells release glycoprotein IFN inducers into the medium (data not shown). It has also been shown that the cell associated inducer can be released from the cell by sonication (6). Since RA could decrease IFN induction by decreasing the level of the cell associated inducer on transformed cells, we measured the effect of RA on the level of inducer that could be released from the cells. This might help differentiate between a simple masking of the inducer on the cell surface and an actual decrease in the amount expressed by the cell. Therefore, L-929 cells were treated with RA for 48 hours, removed from the culture flask, washed extensively, and sonicated to release inducer. Figure 2 illustrates the results of such an experiment. The amount of inducer released by sonication from 10^{-5} M RA treated L-929 cells was decreased by approximately 75% at a 1:3 dilution as compared to controls. This would tend to indicate a decrease in the amount of cell associated inducer due to RA treatment rather than a simple masking of inducer. Similar results were seen with WISH cells (data not shown).

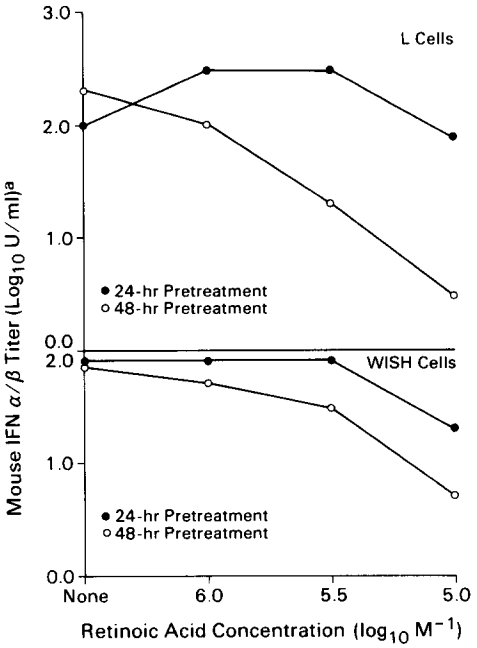

FIGURE 3: Kinetics of RA mediated decrease of IFN inducing potential of L-929 and WISH cells.
L-929 and WISH cells were treated with the indicated concentrations of RA for 24-48 hrs prior to cocultivation with non-sensitized mouse spleen cells.
a) A 0.5 log reduction of IFN titer is significant at a $P \leq 0.05$.

Kinetics of RA mediated decrease of IFN inducing potential of L-929 and WISH cells. Figure 3 illustrates the kinetics of the RA mediated decrease of IFN inducing potential of L-929 and WISH cells. There appears to be a significant time and dose dependent response of the inducer cells to VA. The finding that 48 hours pretreatment of cells is more effective than 24 hours also refutes the possibility that the inhibition is due to a direct masking of the inducer by the RA itself. The time dependency of the RA effect may possibly point to a synthetic or repressional event.

DISCUSSION

Induction of IFN by transformed cells has been shown to be caused by the interaction of a specific lymphocyte receptor with a glycoprotein inducer on the surface of the transformed cell (5,6). Since RA has been shown to modulate the expression of several cell surface molecules (7,8,9,10), this study was initiated to determine the effects of RA

treatment of transformed L-929 and WISH cells on their IFN inducing potential in nonsensitized mouse spleen cells. Dose response experiments indicated that treatment with 10^{-5} M RA produced the greatest inhibition. Both cell associated and solubilized inducers were affected by RA treatment. In addition, kinetics experiments indicated that 48 hours of pretreatment resulted in the greatest decrease. The latter two observations also support the probability that the mechanism of the effect is probably due to a RA mediated decrease and not to a simple masking of the inducer by RA itself.

The observation (1) that transformed cells induce nonsensitized lymphocytes to produce IFN may indicate that the response might represent a natural defense mechanism against tumors. For example, the IFN that is produced may inhibit tumor cell growth (14), or play a role in modulating immune systems (15,16) in the vicinity of the tumor. If this is the case, a number of paradoxical questions are raised since RA treatment of tumor cells decreases their IFN inducing potential while generally inhibiting tumors. Is this RA suppression antagonistic to the possibly beneficial response of IFN production? In addition, since RA restores a more normal phenotype to some transformed cells, would a tumor in effect be held in a quiescent state only to resume growth following cessation of RA treatment? Likewise, would the RA treated transformed cells continue to survive due to lack of immune detection because they exhibited a more normal phenotype? Lastly, the possibility exists that the IFN inducing glycoproteins present on the surface of these transformed cells play a relatively small role in the total antitumor immune response. Taken together, our data indicate a time and dose dependent effect of RA on the ability of transformed L-929 and WISH cells to induce IFNα/β production in non-sensitized lymphocytes. The present findings, when considered with our previous reports (5,6), indicate that the effect is probably due to a decrease in the amount of cell associated glycoprotein IFN inducer(s).

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