Suppression of Interferon Production by Vitamin A

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SUMMARY

Vitamin A (retinoic acid) suppressed interferon production by L cells infected with Newcastle disease virus (NDV). This suppression was maximal when cells were treated with retinoic acid for 2 h after NDV adsorption, indicating that the inhibitory step was an early event. It was not due to inhibition of total RNA synthesis. Retinoic acid treatment caused both a delay in appearance of interferon and a reduced rate of synthesis thereafter.

In the light of the pronounced effect of vitamin A on the induction and synthesis of glycoproteins (De Luca & Yuspa, 1974) and because interferon is an induced glycoprotein (Dorner, Scriba & Weil, 1973), we have examined the effect of vitamin A on interferon production in vitro.

The trans form of retinoic acid (vitamin A acid was obtained from the Sigma Chemical Company, St Louis, Mo). A stock solution was made at 2 mg/ml in dimethyl sulphoxide (DMSO) obtained from the Fisher Scientific Company, Pittsburg, PA. In experiments, a further 100-fold dilution of the vitamin was made in medium, and control cultures received the equivalent final amount (1 %) of DMSO.

Interferon was prepared by treating monolayer cultures of L-929 cells with Newcastle disease virus (NDV), as previously described (Blalock & Gifford, 1975). After adsorption at room temperature for 1 h, residual virus was removed and the cultures were washed and re-fed with fresh medium (Eagle's minimal essential medium – MEM) or medium with vitamin A added. Triplicate cultures were used for each determination, and unless otherwise specified, culture fluids were collected 24 h post infection (p.i.) pooled and clarified by low-speed centrifugation. They were dialysed against pH 2 buffer for 5 days at 9 °C and then against Gey’s balanced salt solution to restore the pH to neutrality.

Interferons were assayed by a plaque reduction assay using vesicular stomatitis virus for challenge as previously described (Blalock & Gifford, 1975). Four dilutions of each sample were each assayed in quadruplicate. Interferon titres are expressed as the amount of an interferon preparation, in µl, that depressed plaque numbers by 50 % (PDD50) as compared to the controls. In our assay system, the NIH mouse reference reagent interferon preparation (code G002–902–026, assigned activity 6000 units) had a titre of 3500 PDD50 units.

In the first experiment, it was found that retinoic acid at 20 µg/ml suppressed interferon production in L cell cultures. The vitamin was added to infected cultures maintained in MEM with 3 % calf serum. After 3 h and after 6 h, groups of cultures were washed to remove the vitamin and DMSO, and were re-fed with MEM and 3 % calf serum. Media were harvested from these and other cultures 24 h after NDV adsorption, and assayed for interferon. As compared with infected cultures not treated with retinoic acid, reductions of 94 %, 94 % and 98 % in the yield of interferon were found in cultures exposed for respectively 3, 6 and 24 h to retinoic acid. Moreover, when replicate non-infected cultures were treated in a similar fashion with retinoic acid and labelled with 10 µCi/ml of 3H-uridine for 1 h at 23 h p.i., as
Table 1. Effect of time of treatment with retinoic acid (20 μg/ml) after NDV adsorption

<table>
<thead>
<tr>
<th>Length of treatment with retinoic acid (20 μg/ml)* (h)</th>
<th>Interferon yield†</th>
<th>PDD₉₀ units/ml</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not added</td>
<td>22,900</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>0-0.5</td>
<td>21,000</td>
<td>91</td>
<td>100</td>
</tr>
<tr>
<td>0-1</td>
<td>15,000</td>
<td>69</td>
<td>100</td>
</tr>
<tr>
<td>0-2</td>
<td>6,000</td>
<td>26</td>
<td>100</td>
</tr>
<tr>
<td>0-4</td>
<td>5,600</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>0-24</td>
<td>5,600</td>
<td>24</td>
<td>100</td>
</tr>
</tbody>
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* 0 time is immediately following adsorption of NDV for 1 h.
† Interferon was determined in the pooled supernates from triplicate cultures.

previously described (Goohra & Gifford, 1971), there was a 74% reduction in ³H-uridine incorporation by those treated for 24 h with retinoic acid but no reduction by cultures treated for only 3 or 6 h. Thus, suppression of interferon production was not correlated with suppression of ³H-uridine incorporation. Part of the reduced interferon yields in this experiment could be explained by inactivation of the newly formed interferon by retinoic acid as previously shown (Blalock & Gifford, 1975). However, 20 μg/ml of retinoic acid in 3% CS would inactivate only about 50% of the interferon. More importantly, in those cultures where the retinoic acid was removed after 3 or 6 h but the interferon was harvested after 24 h, one would not expect any inhibition of interferon activity due to the retinoic acid.

Although the effect on interferon production was apparently not correlated with suppression of ³H-uridine incorporation by retinoic acid, we were concerned that the reduced interferon production might result from some non-specific toxicity of vitamin A. We found that if the concentration of calf serum in the medium was increased from 3% to 10%, treatment for 24 h with retinoic acid at 20 μg/ml did not alter (a) cell numbers, (b) cell viability as determined by trypan blue dye exclusion, (c) the capacity of the cells to allow vesicular stomatitis virus plaque formation, or (d) incorporation of ³H-uridine by the cells. Furthermore, L-929 cells could be seeded and grown successfully in the presence of 20 μg/ml of retinoic acid. These findings indicate that retinoid acid at this concentration was not toxic to the cells if the medium contained 10% calf serum. Thus, in the subsequent experiments, this concentration of serum was used.

The above results indicated that retinoic acid probably suppressed an early event in the production of interferon, since there was no difference in the degree of suppression of interferon yield if the retinoic acid remained in contact with the cells for the 24 h of production or was removed after 3 h. To investigate further the period during which interferon production was sensitive to suppression, retinoic acid and DMSO were removed at various times after NDV adsorption; the cell cultures were washed and replenished with fresh medium without retinoic acid or DMSO. Table 1 shows that maximum suppression of interferon production required 2 h treatment with retinoic acid after NDV adsorption. Again there was no difference in the amount of suppression whether the vitamin A was removed after 2 h treatment or remained on cells for 24 h.

Similar experiments showed that even if addition of retinoic acid was delayed for up to 2 h after the end of NDV adsorption, there was still maximum suppression of interferon production. By 4 h after NDV adsorption, cells were becoming less sensitive to the suppressive effects of retinoic acid, and they were resistant to suppression by 8 h. These data indicate
that retinoic acid affects a step in the production of interferon which maximally occurs about 2 h after NDV adsorption.

When the kinetics of interferon production in MEM with 10 % CS were examined (Fig. 1), interferon was first detected in control cultures 8 h after NDV infection, while interferon was not detected from cultures treated with 20 μg/ml retinoic acid until 4 h later. The apparent rate of synthesis of interferon was reduced in retinoic acid treated cultures, as was the total yield. This difference in apparent rates of synthesis did not result from interference with the process of release of interferon, since we found the same ratio of extracellular/intracellular interferon in both the control and the interferon treated group (data not shown).

Retinoic acid is only one of several biologically active forms of vitamin A. We previously noted a marked difference in the ability of other forms of vitamin A to inactivate pre-formed interferon (Blalock & Gifford, 1975), but all the forms tested suppressed interferon production.

Our findings of in vitro suppression of interferon production by vitamin A is in agreement with a report of an in vivo suppression (Tokumaru, 1967). Based on our previous reports (Blalock & Gifford, 1974, 1975) the first possible explanation was that the suppressive effect might result from inactivation of interferon by vitamin A after it was produced. This was eliminated by several lines of evidence: removal of retinoic acid after 2 h did not cause any less suppression than if it remained present throughout the interferon production period.

Fig. 1. Kinetics of interferon production by control and retinoic acid-treated cells. Cultures infected with NDV as described were then treated for 2 h with retinoic acid (20 μg/ml) in MEM with 10 % CS. Controls were treated with DMSO (1 %). At the times indicated triplicate cultures from each group were frozen at -20 °C, thawed, pooled, dialysed and assayed for interferon. O—O, control, • — •, retinoic acid.
(24 h); interferon production was suppressed in a medium containing calf serum at a concentration (10 %) such that 20 μg/ml of retinoic acid does not inactivate interferon activity; and a form of vitamin A (retinal) which was very effective at suppressing interferon production was ineffective at inactivating interferon activity (Blalock & Gifford, 1975). The enhanced suppression of interferon production observed in medium only containing 5% CS might, however, be accounted for by inactivation of interferon by retinoic acid.

Unimpaired protein synthesis is, of course, a prerequisite to maximal interferon production. Known inhibitors of protein synthesis, such as puromycin and p-fluorophenylalanine, are suppressive throughout the period of interferon production (Buchan & Burke, 1966). If retinoic acid acted by inhibition of protein synthesis it should also be active throughout the cycle of interferon production. Contrary to this, we have shown that addition of retinoic acid 8 h after interferon induction, the time at which interferon is first detectable (Fig. 1), did not suppress the final yield of interferon. Furthermore, cells can proliferate in 20 μg/ml of retinoic acid and VSV can grow normally in vitamin-A-treated cells. Therefore, it seems unlikely that vitamin A acted by inhibition of protein synthesis.

There are several reports concerning the effects of adding actinomycin D at different times after induction of interferon synthesis, and these have shown that interferon messenger RNA synthesis is completed within a few hours following virus infection (Wagner & Huang, 1965; Levy, Axelrod & Baron, 1965; Ho & Breinig, 1965). Although total RNA synthesis was not altered in our system by the addition of vitamin A, the kinetics of the suppressive effect on interferon production are consistent with an effect on interferon messenger RNA synthesis, since the maximum effect occurred about 2 h after adsorption of NDV (Table 1).

It is thus tempting to speculate that vitamin A may specifically repress certain inducible messenger RNAs, one of which may be the interferon messenger RNA. It would seem highly unlikely that all messenger RNA species are suppressed, since L-929 cells can proliferate in the concentration of vitamin A used. If only a few messenger RNA species were suppressed, one would not detect a difference in total RNA. The recent development of a relative quantitative assay for the interferon messenger RNA (Kronenberg & Friedman, 1975) should provide a means for testing whether vitamin A specifically suppresses interferon messenger RNA and thereby controls expression of a specific gene.

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REFERENCES


Short communications


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