Thyroid Hormone Requirement for Retinoic Acid Induction of Mouse Mammary Tumor Virus Expression

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In normal mouse mammary epithelium, insulin, cortisol, and prolactin are absolute requirements for mouse mammary tumor virus expression. Retinoic acid further increased mouse mammary tumor virus expression two- to threefold but only when triiodothyronine was also present; neither retinoic acid nor triiodothyronine alone had any effect.

Many tumor-promoting retroviruses are under direct hormonal control through hormone response elements (HREs) located in their long terminal repeats: both the mouse mammary tumor virus (MMTV) and the human papilloma-virus have glucocorticoid/progesterone HREs (4, 14), the human T-cell leukemia virus has a cyclic AMP HRE (15), and the Moloney murine leukemia virus has a triiodothyronine (T_3)/retinoic acid HRE (17). Since the MMTV also has a sequence that corresponds to a T_3 /retinoic acid HRE, it was of interest to determine if its expression was influenced by either of these hormones.

The mouse mammary epithelium is capable of responding to both hormones. It has a retinoic acid receptor (11), and this hormone will stimulate alveolar development in intact mice (10), DNA synthesis in cultured explants (9), and casein synthesis in cell cultures (16). Conversely, retinoic acid deficiency leads to reduced mammary epithelium and secretory activity in intact mice (6). This tissue is also responsive to T_3 , which stimulates prolactin receptor levels (1). Neither hormone alone has been tested for possible effects on MMTV expression, although one study has reported that retinoic acid has no effect in a mammary tumor cell line when it is added with a phorbol ester (5).

Ovine prolactin (oPRL-17) was kindly provided by the Hormone Distribution Program, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Md., and crystalline porcine insulin (lot 615-08E-220) was a gift from Eli Lilly & Co., Indianapolis, Ind. Cortisol, T_3 , retinoic acid, and HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) were purchased from Sigma Chemical Co., St. Louis, Mo. Medium 199 with Hanks balanced salt solution was obtained from GIBCO Laboratories, Grand Island, N.Y.

Mammary gland explants from virgin mice (C3H/HeN MMTV⁺) were prepared as previously described (8). The explants were cultured on siliconized lens paper in medium 199 containing 20 mM HEPES (pH 7.5), insulin (1 μ g/ml), cortisol (10 ng/ml), prolactin (1 μ g/ml), and T_3 (65 pg/ml), unless otherwise noted. The tissue was incubated under air at 37°C, and the medium was changed daily.

The major MMTV glycoprotein, gp58, was measured by radioimmunoassay, as previously described (2). Briefly, standards or samples were incubated with anti-gp58 antiserum and 125 I-labeled gp58 for 24 h at 4°C. Then, a second antibody was added and the incubation continued for an-

other 24 h. Finally, the pellets were centrifuged through a sucrose cushion, washed twice, and counted.

MMTV RNA was assayed by dot blot hybridization, as previously described (13). Briefly, RNA was extracted from an epithelium-enriched fraction of the explants, spotted on nitrocellulose filters, and hybridized to a 32 P-labeled pMTV-1 probe. The results were quantitated by densitometry of the autoradiograms, which contained internal standards.

The expression of MMTV in normal mammary epithelium requires insulin, cortisol, and prolactin (2, 13); therefore, these three hormones were always present in the medium. The addition of retinoic acid had no further effect on gp58 production; but in the presence of T_3 , a bell-shaped dose-response curve for retinoic acid was obtained (Fig. 1). This phenomenon of inhibition at high concentrations has also been observed for retinoic acid in related systems (9, 16); the maximal stimulation occurred at 325 ng of retinoic acid per ml. In the absence of retinoic acid, T_3 (6.5 to 65 pg/ml) was ineffective (data not shown).

FIG. 1. Effect of retinoic acid on gp58 expression. Mammary gland explants were cultured for 3 days in insulin, cortisol, prolactin, and retinoic acid either with (●) or without (○) T_3 . The free-standing points represent zero concentrations, and the error bars show the standard errors of the means of five separate experiments.

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TABLE 1. Effect of retinoic acid and T₃ on MMTV RNA

Retinoic acid (ng/ml)	T ₃ (ng/ml)	MMTV RNA (ng/100 mg tissue) ^a	P ^b
0	0	3.5 ± 0.5	
0	65	4.0 ± 0.9	NS
325	0	3.5 ± 0.5	NS
325	65	7.3 ± 0.9	<0.005
1,000	65	3.7 ± 0.5	NS
3,250	65	3.9 ± 0.7	NS
350	6.5	6.7 ± 0.9	<0.01

^a Mammary gland explants were cultured for 3 days in insulin, cortisol, and prolactin; retinoic acid and/or T₃ was also present where indicated. The results represent mean values and standard errors of the means from five independent experiments.

^b By the analysis of variance. NS, Not significant versus the control value.

Identical results were seen at the level of MMTV RNA accumulation (Table 1): retinoic acid doubled the amount of MMTV RNA induced with insulin, cortisol, and prolactin, but only if T₃ was also present. Although a full T₃ dose-response curve was not done, concentrations as low as 6.5 pg of T₃ per ml were able to support stimulation by retinoic acid.

In summary, retinoic acid (325 ng/ml) increased MMTV expression two- to threefold, but only in the presence of T₃. This effect is very similar to the one reported by Umesono et al. (18): in their system, an unoccupied T₃ receptor binds a T₃/retinoic acid HRE and blocks retinoic acid induction, thereby making T₃ a requirement for retinoic acid action. This phenomenon would explain the results in both this report and that of Chan and Buehring (5). The latter could not find any effect of retinoic acid on MMTV expression in a mammary tumor cell line, but their cells were cultured in charcoal-stripped serum and no exogenous T₃ was added.

These results are in conflict with the generally held belief that retinoic acid is anticarcinogenic, which might lead one to expect that it would suppress the expression of a tumorigenic retrovirus. However, all previous reports on the protective effects of this compound have involved chemically induced mammary cancers (7, 12). Since retinoic acid is known to be an antioxidant, its anticarcinogenic effects may be more closely related to its chemical properties than to its endocrine activity. The fact that retinoic acid has no effect on naturally occurring mammary tumors supports this hypothesis (10). Alternatively, it may exert its protective effects at another step. Cardiff (3) has proposed that MMTV-induced tumorigenesis occurs in a two-step process; retinoic acid may enhance MMTV expression in the first step but block carcinogenesis in the second step.

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