Thyroid Hormone Modulation of Transformation Induced by Kirsten Murine Sarcoma Virus

Carmia Borek, Augustinus Ong, and Johng S. Rhim

ABSTRACT

We have investigated the effect of triiodothyronine (T₃) on the transformation of normal rat kidney (NRK) cells by the Kirsten strain of murine sarcoma virus (KI-MSV). When NRK cells were grown and infected with KI-MSV in medium lacking T₃, the yield of transformed foci was about one-half that observed in the cultures supplemented with T₃. Individual foci appeared somewhat later in cells grown out in medium devoid of T₃. The yield of KI-MSV released from transformed NRK cells was lower when these cells were maintained in T₃-depleted medium. The results cannot be attributed to cell growth modification by T₃. Normal and KI-MSV-transformed NRK cells grew equally well in monolayer culture in medium containing or lacking T₃. Selective maintenance and removal of T₃ during various phases of the transformation process indicated that T₃ exerted its maximum effect on transformation rates when added to the medium 24 h prior to virus infection. T₃ was less effective in modulating transformation when added simultaneously with virus infection and was ineffective if added 24 h after virus infection.

The results indicate that thyroid hormone is a required factor for optimal transformation by KI-MSV and that the hormone exerts its effects during the early phase of KI-MSV-induced transformation.

INTRODUCTION

The role of hormones at a cellular level during virus-induced neoplastic transformation is relatively unknown.

Cell culture systems offer powerful tools to evaluate such questions in vitro. Our previous studies using hamster embryo cell strains and the cell line C3H/10T1/2 have shown that thyroid hormones are essentially permissive factors for the induction of transformation by X-rays and by chemical carcinogens (3, 4, 6, 7). The removal of T₃ and thyroxine from serum-supplemented media eliminated radiogenic as well as chemically induced transformation in both cell systems without modifying cell survival or cell growth. The addition of T₃ alone was added to the treated serum when assessing the modulating action of thyroid hormone. Thus, in this paper, resin-treated serum is referred to as -T₃, but it should be noted that as in previous reports (4-7), both T₃ and thyroxine have been removed.

The present experiments were undertaken to investigate whether thyroid hormones modulate cellular transformation by an RNA retrovirus, KI-MSV (8, 14, 15). The cell system chosen for these studies was the NRK cell line, which is highly susceptible to transformation by KI-MSV (1, 11).

MATERIALS AND METHODS

Materials. AG 1-X10 resin (chloride form) was purchased from Bio-Rad. T₃ was obtained from Calbiochem-Behring; fetal bovine serum, antibiotics, glutamine, and Eagle's minimal essential medium were obtained from Grand Island Biological Co. Thyroid hormones were removed from fetal bovine serum by adsorption to the resin, as described previously (4, 6, 7) using the method of Samuels et al. (13).

While both thyroxin and T₃ are removed by this procedure (13), T₃ alone was added to the treated serum when assessing the modulating action of thyroid hormone. Thus, in this paper, resin-treated serum is referred to as -T₃, but it should be noted that as in previous reports (4-7), both T₃ and thyroxine have been removed. Stock T₃ (1 mw in 50% 1-propanol) was diluted with medium supplemented with 10% resin-treated fetal bovine serum to give the final T₃ concentration desired. Medium depleted of thyroid hormones was prepared with 10% resin-treated fetal bovine serum to give the final T₃ concentration desired. Medium depleted of thyroid hormones was prepared with 10% resin-treated fetal bovine serum to give the final T₃ concentration desired. Medium containing untreated serum served as a control.

Cell cultures. The established NRK cells were grown and maintained in Eagle's minimal essential medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 1% nonessential amino acids, and gentamicin (50 µg/ml)(11, 12).

Cell growth in media with or without T₃ was determined as described (3) using cells pretreated for 1 week in the appropriate medium.

Virus. The KI-MSV used in these experiments was obtained from the supernatant fluids of a KI-MSV-transformed NRK cell line, No. 58967 (9, 12). Twenty-four-hour culture fluid harvests from No. 58967 cells yielded 3 x 10⁸ FFU of KI-MSV per ml when assayed in NRK cells (12).

Transformation assays. KI-MSV transformation was determined by a focus assay in NRK cells (1, 11, 12, 15). Briefly, NRK cells were preconditioned for 2, 4, or 7 days with medium containing 10% resin-treated fetal bovine serum with or without T₃ (1.0 nm). The cells were seeded at 2 x 10⁵ cells/flask (Falcon Corp.) at 38°C and grown in a humidified incubator with 5% CO₂ and 95% air; 24 h later, the cells were pretreated with DEAE-dextran (25 µg/ml) for 30 min (15) and infected with KI-MSV (50 to 100 FFU/culture). Following infection, the cells were refed with the appropriate medium with or without T₃. Medium was changed twice weekly, and cultures were observed for focus formation. The transformed foci were scored 14 days after infection (1, 10-12, 15).

RESULTS

NRK cells were grown for 2, 4, or 7 days prior to virus infection in media fortified with one of the following sera: untreated; treated with AG 1-X10 resin, which selectively removes thyroid hormones (13) (−T₃); or treated with resin and then fortified with 1.0 nm T₃ (+T₃). The same culture conditions were maintained during and after infection.

The effects of thyroid hormone levels on KI-MSV cell transformation are presented in Table 1 and illustrated in Fig. 1.
Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Serum (T₃ condition)</th>
<th>Time (days) of NRK pretreatment in media containing appropriate serum</th>
<th>KI-MSV (FFU/flask)</th>
<th>Av. no. of transformed foci/flask</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated (euthyroid)</td>
<td>7</td>
<td>0</td>
<td>0</td>
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<td></td>
<td>Untreated (euthyroid)</td>
<td>7</td>
<td>50</td>
<td>35.2 ± 2.2a</td>
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<tr>
<td></td>
<td>Resin treated (−T₃)</td>
<td>7</td>
<td>50</td>
<td>9.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Resin treated +T₃ (+T₃)</td>
<td>7</td>
<td>50</td>
<td>29.0 ± 2.0</td>
</tr>
<tr>
<td>2</td>
<td>Untreated (euthyroid)</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Untreated (euthyroid)</td>
<td>4</td>
<td>100</td>
<td>97.2 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>Resin treated (−T₃)</td>
<td>4</td>
<td>100</td>
<td>45.4 ± 4.2</td>
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<tr>
<td></td>
<td>Resin treated +T₃ (+T₃)</td>
<td>4</td>
<td>100</td>
<td>122.2 ± 6.0</td>
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<td>3</td>
<td>Untreated (euthyroid)</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Untreated (euthyroid)</td>
<td>4</td>
<td>50</td>
<td>15.8 ± 0.6</td>
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<tr>
<td></td>
<td>Resin treated (−T₃)</td>
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<td>50</td>
<td>7.4 ± 0.5</td>
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<tr>
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<td>50</td>
<td>16.2 ± 0.5</td>
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<td>50</td>
<td>42.8 ± 1.7</td>
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<td>Resin treated (−T₃)</td>
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<td>18.4 ± 1.0</td>
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<tr>
<td></td>
<td>Resin treated +T₃ (+T₃)</td>
<td>2</td>
<td>50</td>
<td>32.4 ± 1.6</td>
</tr>
</tbody>
</table>

* Mean ± SE.

a Serum treated with AG1-10X resin which selectively removes thyroid hormones.

b Serum treated with resin and supplemented with 1.0 nm T₃.

The data are based on 4 separate experiments using 2 different virus doses. The average number of transformed foci per flask was approximately one-half in cultures depleted of thyroid hormones (−T₃) compared to the number of foci in T₃-supplemented medium (+T₃) or in control, cultures with untreated serum. A similar inhibition was observed when cells were pretreated for 2, 4, or 7 days prior to viral infection. The reduction in transformation frequency cannot be ascribed simply to an inhibitory effect on cell growth because T₃ depletion did not affect the growth rate or saturation density of normal or KI-MSV-transformed NRK cells (Chart 1), as was also noted in other cell types (3).

The Dependence of KI-MSV-induced Transformation on Thyroid Hormone Concentration. NRK cells were grown for 2 days in medium depleted of or supplemented with various concentrations of T₃. The cells were then infected with 100 FFU of KI-MSV, fed with the same medium, with or without T₃, and examined for transformed foci 14 days later. The data shown in Chart 2 confirm that transformation under T₃-depleted conditions is suppressed, and transformation by KI-MSV is T₃-dependent at concentrations of 10⁻¹¹ to 10⁻¹⁰ and 10⁻⁴ m. Maximum transformation was observed at doses of 10⁻¹⁰ m T₃, a concentration similar to the one required for optimal induction of transformation by radiation and chemical carcinogens (4, 6, 7).

Time Dependence of Thyroid Hormone Action in KI-MSV-induced Transformation. NRK cells were exposed to T₃-depleted medium 24 or 48 h before virus infection, concurrently with virus exposure, and 24 or 48 h after virus infection. In all cases following virus exposure, the T₃-depleted medium remained until the end of the experiment when transformation frequencies were scored.

The results presented in Table 2 indicate that maximum inhibition of transformation occurred when the cultures were rendered hypothyroid 24 or 48 h before virus infection. A lesser degree of inhibition was seen when T₃-depleted medium was added at the time of virus infection, and inhibition was not observed if cells were exposed to medium devoid of T₃ after viral infection despite a continued presence of T₃-depleted medium for the term of the experiment. Thus, the absence of thyroid hormone affects KI-MSV transformation in the early phases of virus-cell interaction, which could include absorption, penetration, and possibly intracellular processing of the virus. However, once virus infection is accomplished in the presence of T₃, the removal of the thyroid hormones has no inhibitory effect on the yield of transformed foci.

Effect of Thyroid Hormone on Production of KI-MSV in NRK Cells. The action of thyroid hormone in modifying the production
THYROID HORMONE MODULATION OF TRANSFORMATION BY Ki-MSV

Chart 1. A, growth of normal NRK cells in medium containing untreated serum (.), serum depleted of T3 (-T3) (.), or serum treated with resin and supplemented with 1 nm of T3 (+T3) (A); B, growth of Ki-MSV-transformed NRK cells in medium containing untreated serum, serum depleted of T3 (-T3), or serum treated with resin and supplemented with 1 nm of T3 (+T3).

Chart 2. Effects of varying concentrations of T3 on transformation induced by Ki-MSV in NRK cells. For transformation experiments, cells were pretreated in media containing various doses of T3 for 2 days prior to Ki-MSV infection (100 FFU/flask) and maintained under the same appropriate medium. The cultures were examined for the appearance of foci after 14 days of incubation, and the fluids of infected cells were harvested and assayed for infectivity in NRK cells (12).

The results shown in Table 3 indicate that a 2-day pretreatment of NRK cells with T3-depleted medium results in a 2-fold reduction in Ki-MSV yield as compared to virus yield in T3-supplemented medium.

Discussion

The present work was designed to extend our previous findings on the effects of thyroid hormones in oncogenic transformation induced by X-irradiation and chemical carcinogens (3, 4, 6, 7). T3 was found to play a crucial role in the induction of transformation by X-irradiation in short-term cultures of diploid hamster embryo cells, as well as in the established line of C3H/10T1/2 mouse embryo cells (6, 7). Furthermore, the same cell cultures are resistant to neoplastic transformation by benzo(a)pyrene or by N-methyl-N-nitro-N-nitrosoguanidine when T3 is removed from the culture medium (4). T3 was found to exert its maximum effect when added 12 h prior to treatment with X-irradiation or chemical carcinogens. It should be noted that...
depletion of the culture medium thyroid hormones did not alter the cell growth or the survival of the treated cells (3, 4, 6, 7). The results showed that the effects of the thyroid hormones are exerted during the initiation of transformation and are seemingly mediated by the synthesis of host protein(s) that play(s) a key role in neoplastic transformation, both by chemical carcinogens and X-irradiation (4, 6, 7).

The present experiments extend the work to studying the effects of thyroid hormones on neoplastic transformation induced by Ki-MSV (8, 14, 15) in NRK cells (1, 11).

Transformation was inhibited in cultures exposed to Ki-MSV under thyroid hormone-depleted conditions (Table 1; Fig. 1). Omission of T3 during the entire duration of the experiment resulted in a 2-fold reduction in the number of transformed foci as compared to that observed in cultures continuously provided with thyroid hormone. When cultures infected under T3-depleted conditions were kept for 4 weeks postinfection (results not shown) transformation rates were as low as those in cultures maintained for 2 weeks following infection indicating a true suppression of transformation. The yield of Ki-MSV released from infected NRK cells was also greatly reduced (Table 3).

The inhibition of transformation and the lowered virus yield under T3-depleted conditions cannot be ascribed to an inhibitory effect on cell growth because T3 did not affect the growth rates or saturation densities of normal and Ki-MSV-transformed NRK cells (Chart 1).

T3 is therefore required for optimal transformation by Ki-MSV. The addition of T3 at various concentrations to T3-depleted medium resulted in transformation frequencies by Ki-MSV which were T3 dose dependent (Chart 2). Maximum transformation was observed when T3 was added at 10^{-10} M, a dose level similar to the one required for maximum transformation induced by X-rays, benzo(a)pyrene, and N-methyl-N-nitro-N-nitrosoguanidine.

Thyroid hormone appears to be most effective during the early transformation process by Ki-MSV, i.e., 2 to 3 days before viral infection (Table 2). The requirement for T3 and the critical timing for its action are strikingly similar for transformation by Ki-MSV, a retrovirus (2, 8, 15, 16), and for adenovirus type 5 (5).

Thyroid hormone appears therefore to serve as a permissive physiological factor in virus transformation, and its action is exerted at early stages of virus-cell interaction, i.e., during the initiation of transformation processes. The mechanism of T3 action in murine sarcoma virus transformation is unknown. It is possible that thyroid hormone is necessary for optimal virus absorption and penetration; the hormone may also play a role in the intracellular processing that follows, including early expression of viral genes. Further analytical work is required to establish these points and to evaluate alternate explanations for the phenomena described here.

The findings reported here and those observed previously (4–7) highlight the importance of thyroid hormones in carcinogenesis. They indicate that thyroid hormones play a critical role in modulating cellular neoplastic transformation induced by a wide spectrum of oncogenic agents. These include radiation, whose oncogenic actions are imparted to cells within a fraction of a second (3), chemical carcinogens that act directly and those that require metabolic activation for neoplastic transformation, and viral oncogenic agents.

The system presented in this report on cellular transformation by Ki-MSV and the results observed offer possibilities to further elucidate at a cellular and molecular level the regulation of retroviral cell transformation which is so critically dependent on the presence of thyroid hormones.

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**REFERENCES**

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