

ON THE CELLS OF ORIGIN OF RADIOGENIC THYROID CANCER:
NEW STUDIES BASED ON AN OLD IDEA^{*,+}

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*Project is supported by U.S. Department of Energy grant # DE-FGO2-87ER60507 to KHC.

⁺Prepared for publication in the Journal of Radiation Research (Japan) in the proceedings of the Japan Radiation Research Society International Symposium on "Radiation Carcinogenesis in the Whole Body System", Tokyo, 4-7 December 1990.

ABSTRACT

We have presented evidence that the functional thyroid follicles (follicular units, FU) which are formed in grafts of monodispersed rat thyroid cells, and hence the thyroid tumors which later develop in such grafts, are clonal in origin. Transplantation assays indicate that the clonogens comprise ~1% of the cells in monodispersed suspensions of normal thyroid tissue. Carcinogenesis studies show that neoplastic initiation of thyroid clonogens by radiation is a common event. Promotion-progression to cancer from radiation initiated clonogens has, however, been shown to be inversely related to the total grafted thyroid cell number; i.e. more tumors develop per irradiated clonogen in grafts of small cell numbers than of large cell numbers. Recent studies have been designed to investigate: a) whether the cell number-dependent inhibition of promotion-progression is mediated by remote hormonal feed-back, local cell-cell interactions, or both; b) the cell population kinetics of the clonogen subpopulation during goitrogenesis and goiter involution; and c) the effect of prolonged exposure to high levels of TSH (thyrotropin) on the capacity of the clonogens to give rise to functional FU. The results indicate that local cell-cell interactions play an important role in the cell number-dependent suppression of neoplastic promotion-progression. They also show that if sufficient thyroid cells are grafted, the thyroid-pituitary axis can be reestablished in thyroidectomized rats fed normal diets. In such animals given iodine deficient diets, the FU that develop in the thyroid grafts shift their secretory pattern to increase the ratio of T3 (triiodothyronine) to T4 (thyroxine), and thus conserve the

available iodine. Finally, the clonogenic subpopulation is conserved during both goitrogenesis and goiter involution. When they are transplanted to thyroidectomized recipients, clonogens from two types of goiters form FU that are morphologically indistinguishable from those that develop in grafts of normal thyroid clonogens. Furthermore, the secretion of T3 and T4 by such grafts is dependent on the grafted clonogen number, and hence FU formation, and not on the total number of thyroid cells transplanted. We conclude that the thyroid clonogens, the presumptive cancer progenitor cells, have many of the characteristics of stem cells.

INTRODUCTION

A major effort in our laboratory has been devoted to studies of the origins of radiogenic and hormonally-induced cancer at the cellular level *in vivo*. These studies were initially undertaken to further test the frequently stated hypothesis that the most likely target cells for cancer induction are cells with stem-like characteristics, i.e. cells characterized by a capacity for self renewal and a high future proliferative and differentiative potential (see Pierce et al. 1978; Buick and Tannock 1987). We chose to attempt to measure and, if possible, characterize such an epithelial cell sub-population in rat thyroid glands. The thyroid is susceptible to radiogenic cancer in rats as well as in humans (Dumont et al. 1980; Clifton 1986).

Hypophyseal TSH (thyroid stimulating hormone) is a potent mitogen for normal thyroid cells and an important promoter of neoplasia in radiation initiated thyroid cells

(Dumont et al. 1980). The secretion of TSH is increased in response to decreases in titers of the thyroid hormones T₄ (thyroxine) and T₃ (triiodothyronine). In the current studies, we have taken advantage of both the mitogenic and neoplasm-promoting effects by experimentally inducing elevated TSH levels.

Functional thyroid follicles develop at the sites of grafts of monodispersed rat thyroid cells in the fat pads of syngeneic thyroidectomized recipients under the influence of TSH (Clifton et al. 1978). A terminal dilution transplantation assay which utilizes these multicellular FU (follicular units) as the endpoint has been developed to measure the colony-forming capacity of thyroid cell suspensions under different physiological conditions (DeMott et al. 1979; Clifton and Gould 1985). With this assay, it has been demonstrated that: a) the cell dose-FU formation response follows that to be expected if each FU were clonal in origin, i.e. arose from a single cell; b) the results of morphological studies during their formation also are consistent with the clonal origin of FU; c) the radiation dose-FU formation response and potentially lethal damage repair capacity follow the relationship and kinetics to be expected if FU are clonal structures; and d) radiogenic cancer can develop within FU in TSH-stimulated graft sites (reviewed in Clifton 1990). The term *clonogen* has thus been applied to the cells which give rise to FU and are the presumptive progenitor cells of cancer in thyroid cell grafts. Transplantation assays indicate that there is about one clonogen per 50 to 150 cells in monodispersed suspensions prepared from whole young male rat thyroid glands (Watanabe et al. 1988).

Data from carcinogenesis experiments have illustrated that cancer initiation by radiation is a highly frequent event at the clonogenic cell level (Mulcahy et al. 1984). Furthermore, carcinoma development in thyroid cell graft sites bears a complex relationship both to the number of clonogens present immediately after irradiation and to the hormonal milieu (Watanabe et al. 1988). Thus it is important to further define the physiology and population kinetics of the thyroid clonogens under different hormonal conditions both *in situ* and following transplantation. In this report, we will briefly summarize recent data a) on local cell-cell and remote hormonal feedback interactions during neoplastic promotion of initiated cells among the progeny of grafted clonogens in multicellular FU, b) on clonogenic cell population kinetics *in situ* during goitrogenesis and goiter involution, and c) on the reestablishment of the thyroid-hypothalamus-pituitary hormonal feedback system in thyroid cell-grafted thyroidectomized rats and its dependence on the formation of FU by the grafted clonogens. The results of these studies support the conclusion that the thyroid gland contains a small sub-population of clonogenic epithelial cells which possess many stem cell-like characteristics. These clonogens respond differently to hormonal manipulations than the bulk of the thyrocytes, are preferentially conserved in different hormonal situations, and are susceptible to radiogenic malignant initiation. In the thyroid, as in the mammary glands in comparable experiments, cancer appears to be a disease primarily of the least differentiated cells with the highest proliferative potentials.

MATERIALS AND METHODS

Animals: Male Fischer (F-344) rats, specific pathogen free (Harlan Sprague Dawley, Madison, WI or obtained from a breeding colony derived from Harlan Sprague Dawley stock) were employed in these studies. The animals were housed at $76\pm 4^{\circ}\text{F}$ and 50% relative humidity with a 12 hour light-12 hour dark cycle. Goitrogen treated and age-matched control rats were maintained in plastic cages on autoclaved pine shavings; transplant recipients were maintained in wire bottom cages.

All animals were four weeks (carcinogenesis study) or five weeks old (other studies) at the beginning of the various treatments or at the time of transplantation. They were provided with drinking water and chow *ad libitum*. Surgical procedures and blood collections were performed under light ether anesthesia and the rats were killed by ether overdose. To maximize the efficiency of FU formation following transplantation, recipient animals were surgically thyroidectomized (tx) before grafting (Mulcahy et al. 1980).

Cell Monodispersion and Transplantation for Assays of Clonogen Concentration: The technique for enzymatic monodispersion and transplantation of thyroid cells has been described (Clifton and Gould 1985). Briefly, donor animals were killed and the goiters or control thyroids were bluntly dissected from the trachea, pooled, and minced. The minced tissue was then incubated with shaking in α -Minimum Essential Medium (α MEM, GIBCO, Grand Island, NY) containing collagenase Type III (300 units/ml, Worthington, Freehold, NJ) for 2 h at 37°C . The digested tissue was pelleted and resuspended in α MEM containing

pronase (1.25%, Calbiochem-Behring, La Jolla, CA) and incubated with shaking at 4°C for 90 min. The digests were then passed through 53 μm nylon filter, pelleted, washed, and resuspended in αMEM containing DNase Type I (25 μg/ml, Worthington, Freehold, NJ). The concentrations of intact cells were determined by trypan blue exclusion and visual counting with a microscope and hemacytometer.

Appropriate cell dilutions were prepared (typically, 6-7 serial 1:1 dilutions) and aliquots of each suspension mixed with an equal volume of 50% W/V brain homogenate such that the desired number of intact cells could be injected in a volume of 60 μl. Each recipient received five injections: one into the intrascapular subcutaneous white fat pad and two into each of the inguinal subcutaneous fat pads. All five sites in a single recipient were injected with inocula from the same cell dilution group. Each cell dose was inoculated into three to four animals yielding 15-20 sites which could be evaluated. At least four weeks after inoculation, the recipient animals were killed and the fat pads were excised, mounted on glass slides, fixed, stained, cleared, and examined under a dissecting microscope for the presence of FU.

The fraction of transplant sites within each cell dilution group which contained at least one FU and the number of intact cells injected into each site of that group were computer fit to the model of Porter et al. (1973) which states that:

$$1 - P_s = \exp(-kz^2)$$

where P_s is the probability of one or more FU, z is the number of intact cells injected, k is

the clonogenic fraction, and s is the slope of the relationship between the number of scored clonogenic cells within an inoculum and inoculum size. Estimates of k and s were generated from the data with a computerized iterative method using a maximum likelihood procedure and were then used to calculate an FD50 (follicular dose-50%) value, i.e. the average number of cells required to produce one or more FU in 50% of the transplant sites. The FD50 is inversely related to the clonogenic fraction.

Carcinogenesis Study: Thyroid glands were removed from donor rats and monodispersed as described above for the clonogen assays. A portion of the cell suspension was removed for sham irradiation and the remainder was exposed to 5 Gy irradiation at a rate of 7.1 Gy/m in a ^{137}Cs gamma radiation source. Following irradiation the cell concentration was adjusted and serial dilutions were prepared to yield the desired number of surviving irradiated clonogenic cells per inoculum. Rat brain homogenate was added to each inoculation suspension to a final concentration of 25% wt/vol. The recipients, one day before, were divided among groups which received either irradiated cells or control cells. Each of the four serial cell dilutions to be assayed was injected into one of four s.c. fat pads of each animal, thus assuring that all recipients obtained the same total surviving clonogenic cell number (Table 1). Twenty replicate experiments were performed to accumulate 90-100 rats in each of the experimental groups. The data from all replicates were pooled for statistical analysis. Following grafting, recipients were maintained on low iodine diet to promote the expression of thyroid tumors and acidified water supplemented with 10.0 g/l

calcium lactate, 22.6 g/l glucose, and 32.5 mg/l sodium hypochlorite (calcium water) to prevent tetany. When a s.c. nodule reached 10x10 mm it was scored as a gross tumor was surgically resected, and the animal was returned to the study. Animals which died during the course of the study were examined for tumors at necropsy and censored on the day of death. Results were corrected for intercurrent loss by the procedure of Kaplan and Meier (1958). Final histopathological and some other analyses are not yet completed. The current analyses are based on gross tumor incidences and latencies. Gross tumor latencies are expressed in terms of the restricted means which quantify the average times to gross (10x10 mm) tumors in those rats that developed gross tumors before a given time (Miller 1981). The restricted means were calculated by integrating the areas beneath the Kaplan-Meier curves from day 0 to day 665.

Serum Hormone Radioimmunoassays: For determination of serum hormone levels, blood was collected by retroorbital puncture or by pipet from a severed brachial artery, allowed to clot, and the serum was frozen at -70°C until assay. TSH levels were determined by radioimmunoassay with reagents provided by National Institute of Diabetes and Digestive and Kidney Disease (RP-2 standards, rTSH-I-8, and anti-rTSH S-5). Total T₄ levels were determined with a commercially available antiserum and standards (SIGMA Prod. Nos. T2652 and T2376 respectively), the manufacturer's recommended protocol (antiserum insert dated 9/87), and [¹²⁵I]-T₄ (Prod. No. NEX-111X, NEN, Boston, MA). Total T₃ levels were determined also using a commercially available antiserum and standard (SIGMA Proc. Nos.

T2777 and T2877 respectively), the manufacturer's recommended protocol (antiserum insert dated 12/87), and [¹²⁵I]-T₃ (Prod. No. NEX-110X, NEN, Boston, MA). For T₃ and T₄ determinations, standards were prepared in hormone free rat serum obtained from animals six weeks after surgical thyroidectomy. The lower limits of detection for the TSH, T₃, and T₄ assay systems were approximately 0.5, 0.1, and 1.0 ng/ml respectively.

Goitrogen Treatment: Two different goitrogenic regimens were employed. One group of rats, designated ATA/ND, was supplied with 0.05% ATA (3-amino-1,2,4-triazole, SIGMA, St. Louis, MO) in the drinking water and a "normal diet" (ND) of Breeder Blox (Wayne, Bartonville, IL). A second group, designated KClO₄/LID, was supplied with 0.5% KClO₄ (Aldrich, Milwaukee, WI) in the drinking water and Remington low iodine diet (<50 ng I/gm diet, TEKLAD, Madison, WI). The goitrogens inhibit thyroid hormone synthesis at two different biochemical loci. ATA is a potent thyroperoxidase inhibitor (Strum and Karnovsky, 1971); KClO₄ competitively inhibits the cellular uptake of iodide (Green 1986). Both goitrogens were prepared weekly in deionized water. Age-matched control animals and transplant recipients were maintained on ND and calcium water (see above).

For the experiment on the process of goiter involution, animals which had been treated with ATA/ND for 12 weeks were switched to the same regimen as the age-matched control rats. The clonogen concentration of cells from control glands and from goiters were determined at three or six week intervals during goiter growth, the plateau phase, and goiter involution.

Total thyroid clonogens were estimated from the clonogenic fractions and total thyrocytes calculated from total thyroid DNA (Wannemacher et al. 1964) assuming 6.5×10^{-6} μg DNA/cell (Wollman and Breitman, 1970).

FU Formation and Thyroid Function: Restoration of thyroid function in tx recipients grafted with 2.4×10^3 to 3.0×10^5 normal thyroid cells was determined by sequential serum hormone analyses. Thyroid cell grafted recipients, intact, and tx control animals were maintained on either ND or LID and calcium water. All animals were bled weekly by retroorbital puncture and serum T3, T4, and TSH levels were determined as described above.

On the basis of the clonogenic fractions determined by transplantation assay, ~ 10 , ~ 80 , and ~ 640 clonogenic control, 6 week ATA/ND, or 6 week KClO_4 /LID cells were transplanted per animal into tx -14 recipients. Due to the large number of total cells required to contain 640 KClO_4 /LID clonogens, the recipients of all three types of cells recieved two injections of 100 μl of each cell dilution per transplant site. Four recipients were injected per cell dose. Four tx -14 animals were sham injected with brain homogenate alone. Four intact, age-matched animals were also included. The animals were bled eight weeks after transplantation; and serum T3, T4 and TSH levels were determined as described above. The fat pads from all grafted recipients were scored for FU.

RESULTS

Cell-cell Interactions During Thyroid Cancer Promotion: In previous carcinogenesis

studies, we demonstrated that the frequency of overt cancer per grafted irradiated thyroid clonogen was inversely related to the numbers of surviving cells grafted per site in thyroidectomized recipients maintained on LID (Mulcahy et al. 1984). That is, as the number of grafted thyroid cells was increased, the efficiency of promotion to neoplasia was decreased. Moreover, the addition of unirradiated thyrocytes to graft inocula of irradiated cells further depressed promotion-progression to cancer (Watanabe et al. 1988). Preliminary assays of sera from thyroidectomized rats that had received large or small numbers of grafted thyroid clonogens had suggested that the FU which developed at the sites of large grafted clonogen numbers could secrete sufficient thyroid hormones to cause feed-back inhibition of TSH secretion; TSH levels remained elevated in the recipients of the smaller cell number grafts. Hence, TSH-related promotion would be expected to be more intense in rats grafted with the smaller cell numbers than in those grafted with larger cell numbers (Watanabe et al. 1988).

The large carcinogenesis study discussed herein was designed to determine whether the grafted cell number-dependent suppression of neoplastic promotion is mediated through changes in TSH secretion by the anterior pituitary, results from local cell-cell interactions, or both. Although the experiment is not completed, some of the data are adequate for preliminary analysis. By ~ 670 days after grafting, as compared to the unirradiated control series C, the 5 Gy gamma ray exposure of the cells grafted in series R increased the gross tumor frequencies and reduced the tumor latencies in the graft sites at all surviving clonogen

doses (Table 1). As in previously reported experiments, however, over the 27 fold differences in grafted clonogen numbers, i.e. from ~ 3.3 to ~ 90 clonogens per site, there were only eightfold to tenfold increases in the frequencies of gross tumors. These results reflect a progressive decrease in the efficiency of tumor formation per surviving grafted clonogen as the number of clonogens per graft site was increased.

In this experiment, animals of series CE and RE were grafted at four sites with the same numbers of unirradiated or of irradiated clonogens as groups C and R respectively. In addition, however, each rat also was grafted at a fifth site with 1400 unirradiated clonogens. The addition of the large number of clonogens at the fifth graft sites of series CE and RE did not significantly alter the latencies or numbers of tumors in the unirradiated or irradiated test-cell sites as compared to those in series C and R, respectively (Table 1).

An experiment performed concurrently was designed to investigate the effects of grafts of 0 or of 2.4×10^3 to 3.0×10^5 thyroid cells per rat on T3, T4 and TSH concentrations after transplantation to thyroidectomized animals fed ND (normal laboratory chow) or on LID (low iodine diet) (Domann et al. 1990). In rats fed either ND or LID, serum T3 and T4 were below detectable levels one week after transplantation (data not shown). By three weeks after grafting, T3 titers were near or within the normal range in rats that had received 3×10^5 or 6×10^4 total thyroid cells and were fed ND (Table 2). T3 and T4 levels did not reach normal during the experiment in rats that were fed ND and received lower grafted cell numbers. TSH titers had begun to fall toward normal by six weeks after grafting of the

highest cell dose (Table 2), and seven weeks after grafting of the second highest (data not shown).

In rats fed LID, T4 titers remained severely depressed throughout the experiment (Table 2). In contrast, despite iodine restriction, T3 titers reached near normal by the sixth week in those rats that had received the two highest cell doses (Table 2). TSH titers had begun to approach the norm by seven weeks after grafting in the rats that had received the highest cell dose, but remained elevated in the other cell dose groups throughout the experiment (data not shown).

These results have been compared with other less detailed studies of serum hormone levels in thyroidectomized grafted rats. It is clear that the degree of restoration of thyroid function in such rats is dependent on dietary levels below the 50 ng iodide per g food which was the minimum measurable value with the technique used in the analysis furnished by the supplier of the diet. Different batches of LID from the same supplier supported different levels of thyroid function in such rats.

Total Cell Population and Clonogen Sub-population Kinetics During ATA/ND-induced Goitrogenesis and Goiter Involution: Rapid goitrous growth with a marked increase in total thyroid DNA occurred in response to the rapid increase in TSH levels during the first six weeks of ATA/ND treatment of intact young rats. Thyroid size then stabilized or increased slowly in the plateau phase goiters during the next 12 weeks of treatment (Fig. 1) (Groch and Clifton 1990a).

Transplantation assays showed that although the total number of thyrocytes increased about 30 fold, the number of clonogens per thyroid gland remained essentially constant during the six week period of rapid ATA/ND-induced goitrous growth (Fig. 1). Thereafter, during the plateau phase, the total number of clonogens per gland increased about six fold (Groch and Clifton 1990a).

When the ATA/ND regimen was stopped after 12 weeks, a rapid decrease in TSH titers occurred. Consequent extensive loss of thyrocytes and thyroid DNA resulted in involution of the goiter. The total number of clonogens per gland was not decreased, however, indicating preferential loss of non-clonogenic cells and preferential conservation of the clonogen sub-population (Fig. 1).

Dependence of Thyroid Hormone Secretion on Grafted Clonogen Numbers Rather than Total Grafted Cell Numbers: The clonogenic fractions of untreated control thyroids and of early plateau phase goiters from rats that had been treated with either ATA/ND or $KClO_4/LID$ were determined by transplantation bioassay. Groups of thyroidectomized rats were then grafted with total thyroid cell numbers which were estimated to contain ~ 10 , ~ 80 or ~ 640 clonogenic cells from each of these types of glands per graft inoculum (Groch and Clifton 1990b). About 17 times as many total ATA/ND-induced goiter cells, and ~ 63 times as many $KClO_4/LID$ -induced goiter cells, were required to contain the same estimated numbers of clonogens as in suspensions of cells prepared from normal glands (Table 3).

Eight weeks after transplantation, the titers of T3, T4 and TSH were determined in

blood samples from the recipient animals. As expected, T3 and T4 levels were found to be increased, and TSH titers were found to be decreased, in proportion to the total numbers of grafted cells from normal glands or from either goiter type (Table 3). Despite the large differences in total numbers of cells grafted from the normal glands and from the two goiter types, when the hormone levels were expressed in terms of the numbers of clonogens grafted, the source of the grafted clonogens made essentially no difference. That is, the glandular structures that had developed from graft inocula containing ~ 10 , ~ 80 or ~ 640 clonogens from either goiter type produced nearly the same T3 and T4 levels as the glandular structures that arose from grafts of ~ 10 , ~ 80 or ~ 640 clonogens from normal thyroid glands, respectively (Table 3). Furthermore, these structures were indistinguishable in size, number of follicles, and general morphology in sites which had received comparable clonogen numbers irrespective of the treatment of the cell donors (Groch and Clifton 1990b).

DISCUSSION

Preliminary analysis of gross tumor incidences and latencies in the thyroid graft sites of the large carcinogenesis experiment are in accord with the conclusion that local cell-cell interactions play a major role in grafted cell number-dependent suppression of neoplastic promotion-progression in grafts containing radiation-initiated thyroid clonogens. This carcinogenesis study was designed such that each thyroidectomized recipient rat in series C and R received a total of ~ 133 surviving unirradiated or irradiated clonogens, respectively.

It was reasoned that any changes in TSH secretion by the pituitary gland caused by T3 and T4 secretion by FU in the graft sites would affect all graft sites equally; grafts which initially contained about three surviving clonogens would be subjected to the same level of TSH-mediated neoplastic promotion as grafts that initially contained 10, 30 or 90 clonogens. Despite this equalizing of TSH effects, our preliminary analysis shows a progressive decrease in the incidences of gross tumors per grafted irradiated clonogen as the number of clonogens transplanted per site was increased. The effect of transplanted cell number was less in grafts of unirradiated clonogens. These findings illustrate local cell number-dependent suppression of neoplastic promotion-progression of radiation-initiated thyroid cells to overt malignancy. This phenomenon may be comparable to the disappearance of carcinogen-exposed preneoplastic tracheal epithelial cells when grafted in the presence of normal epithelium (Terzaghi-Howe 1987), and to the cell density independent frequency of morphological transformation in irradiated cultured C3H 10T1/2 cells (Kennedy et al. 1980).

The transplantation of 1400 thyroid clonogens to a fifth graft site in each rat of series CE and RE was done to allow for separate analysis of the effects of thyroid hormones produced by the progeny of this large number of grafted clonogens on tumor progression in the grafts of smaller cell numbers. The preliminary analysis suggests that the large fifth graft had no clear effect on tumorigenesis in the other grafts of irradiated or unirradiated clonogens.

T3 and T4 secretion by thyroid cell grafts depends on the development of FU and

hence on grafted clonogen number, not on the total number of grafted thyrocytes. When the total number of clonogens grafted is adequate, the thyroid-hypothalamus-pituitary hormonal feed-back axis can be reestablished in thyroidectomized animals. Moreover, under conditions of restricted dietary iodine intake in such rats, the thyrocytes in the grafts increase the ratio of T3 secreted to that of T4, and thereby increase the efficiency of utilization of the available iodine. This change in secretory pattern would appear to have adaptive value (Domann et al. 1990).

The thyroid clonogens are characterized by their high proliferative potential, the preservation of their sub-population, and by their ability to give rise to functional follicles by proliferation and differentiation. This latter capacity is not altered by massive glandular hyperplasia during exposure to goitrogens; moreover, during the rapid cell loss which occurs during the involution of goiters, the sub-population of clonogens is preferentially preserved. These latter observations suggest that the sub-population of clonogenic thyrocytes has evolved to play an important role in the normal and pathological physiology of the thyroid gland. Their high proliferative potential makes the clonogens the most likely precursor cells in the development of cancer. Although it is unknown whether the thyroid clonogens are stem cells, they have many stem cell-like attributes. It seems likely that most if not all carcinomas will be found to arise from such preferentially preserved sub-populations of cells with high proliferative potentials.

ACKNOWLEDGEMENTS

The authors are indebted to Mrs. Joan Mitchen for excellent technical assistance, and to Mrs. Jane Barnes, Mrs. Dawn Church and Mr. Nam Deuk Kim for their help when required. Mrs. Peggy Ziebarth was a great aid with the preparation of the manuscript.

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Table 1: Preliminary analysis of tumor data, carcinogenesis experiment.

<u>Group</u>	<u>Clonogens per Site**</u>	<u>Gross Tumors*</u>		
		<u>Incidence, Percent</u>	<u>Latency, Days</u>	<u>Incidence/10³ Clonogens</u>
C	90	38	616	4
	30	27	635	9
	10	15	644	15
	3.3	5	647	16
R	90	78	506	9
	30	54	583	18
	10	26	626	26
	3.3	14	625	42
CE ⁺	90	46	626	5
	30	20	641	7
	10	17	643	17
	3.3	14	663	43
RE ⁺	90	79	557	9
	30	39	612	13
	10	22	629	22
	3.3	15	639	45

*Palpable masses scored as tumors when they reached 10x10 mm in mean diameter.

**Sites rotated from rat to rat during grafting such that all cell inoculum sizes were grafted in equal frequencies in all site locations.

+A fifth site in each rat was grafted with 1400 unirradiated clonogens.

Table 2: Effect of cell dose and diet on the reestablishment of the hypothalamic-pituitary-thyroid axis in thyroidectomized/thyroid cell grafted rats (Domann et al. 1990).

<u>Week</u>	<u>Diet</u>	<u>No. Grafted Cells per Rat</u>	<u>Hormone (ng/ml)</u>		
			<u>T3</u>	<u>T4</u>	<u>TSH</u>
3	ND	intact*	0.59±0.18	30.6±3.6	3.8±1.1
		300,000	0.41±0.11	24.5±3.7	32.4±6.8
		60,000	0.34±0.19	14.1±1.7	44.6±10.4
		12,000	<0.12	<2.0	46.9±9.4
		2,400	<0.12	<2.0	44.8±7.7
		0	<0.12	<2.0	52.7±7.6
6	ND	intact	1.08±0.26	30.5±8.4	2.9±0.4
		300,000	0.62±0.04	33.9±3.4	9.1±3.1
		60,000	0.55±0.05	26.7±3.6	16.4±6.8
		12,000	0.37±0.14	10.4±2.2	54.1±15.6
		2,400	<0.12	<2.0	43.4±10.5
		0	<0.12	<2.0	45.6±3.6
3	LID	intact	0.61±0.06	11.5±3.4	6.5±1.6
		300,000	0.26±0.08	<2.0	43.4±3.2
		60,000	<0.12	<2.0	43.1±9.9
		12,000	<0.12	<2.0	55.3±6.7
		2,400	<0.12	<2.0	44.2±7.0
		0	<0.12	<2.0	51.7±6.0
6	LID	intact	0.78±0.08	5.7±0.9	6.8±0.8
		300,000	0.53±0.16	5.4±0.2	26.4±9.3
		60,000	0.49±0.11	6.1±0.2	34.4±0.6
		12,000	<0.12	<2.0	51.5±9.6
		2,400	<0.12	<2.0	43.0±6.7
		0	<0.12	<2.0	41.2±5.0

*Intact indicates not thyroidectomized. 0 indicates thyroidectomized but not grafted with thyroid cells.

Table 3: Dependence of T3, T4 and TSH titers in thyroidectomized rats on total grafted clonogens independent of the treatment of clonogen donors (Groch and Clifton 1990b).

<u>Cell Source</u>	<u>Cells per Animal</u>	<u>Clonogens per Animal</u>	<u>TSH (% tx)*</u>	<u>T4 (% intact)+</u>	<u>T3 (% intact)+</u>
Control	96,000	640	13	99	55
	12,000	80	63	39	24
	1,500	10	74	4	11
ATA/ND	1,600,000	640	10	88	60
	200,000	80	68	39	35
	25,000	10	82	14	14
KClO ₄ /LID	6,000,000	640	14	107	78
	750,000	80	62	40	36
	93,750	10	80	12	21

*TSH titers as percentages of those in ungrafted thyroidectomized ("tx") rats.

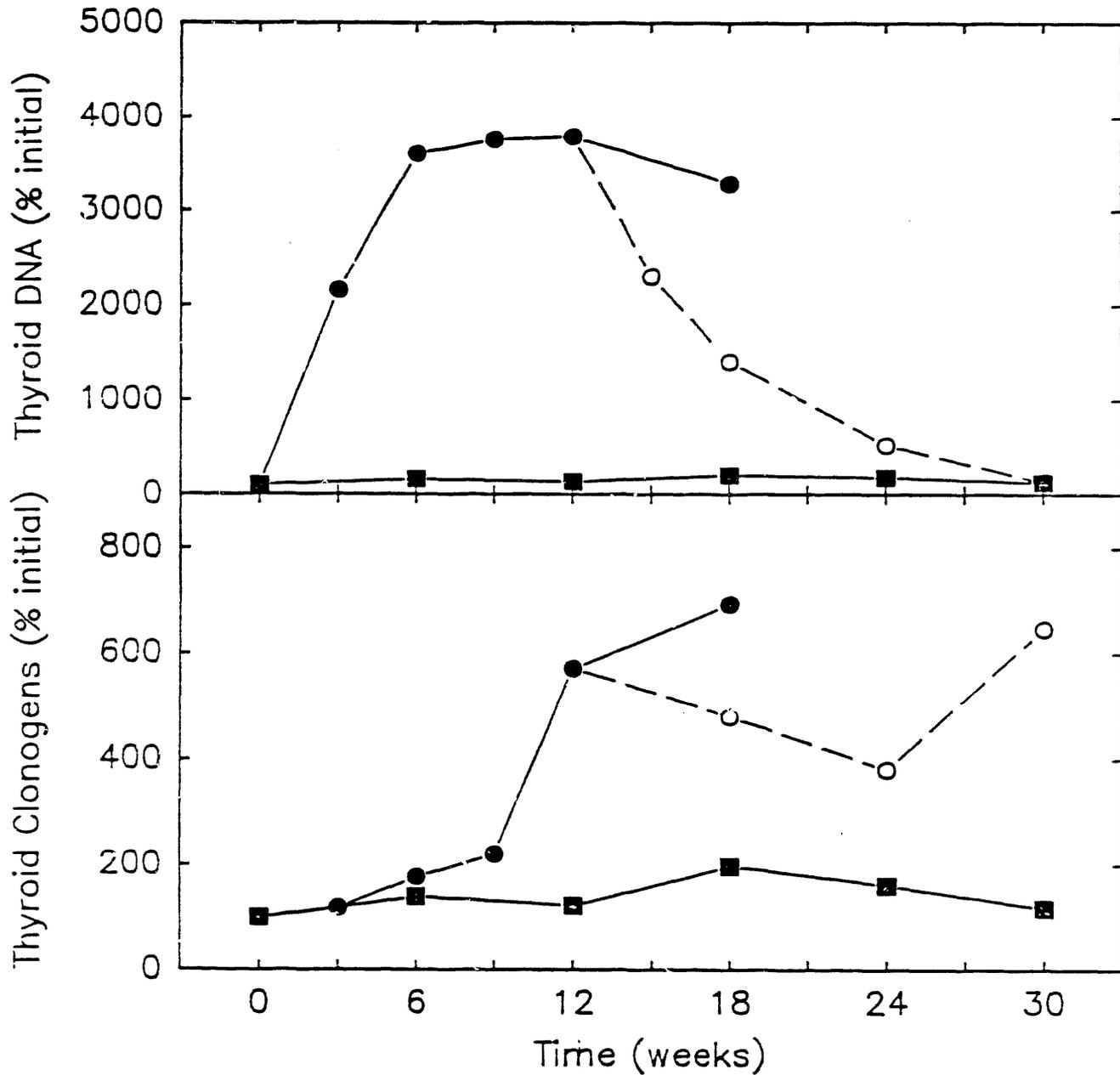
+T4 and T3 titers as percentages of those in intact rats.

FIGURE LEGENDS

Figure 1. Effect of ATA/ND-induced goitrogenesis and goiter regression on total thyroid DNA (upper) and total thyroid clonogen content (lower) expressed as percent of the initial value. Solid squares and line: untreated control values; solid circles and line: values from rats placed on ATA/ND at time 0; open circles and dashed line: values from rats treated with ATA/ND for 12 weeks and then returned to the normal regimen (Groch and Clifton 1990a).

FIGURE LEGENDS

Figure 1. Effect of ATA/ND-induced goitrogenesis and goiter regression on total thyroid DNA (upper) and total thyroid clonogen content (lower) expressed as percent of the initial value. Solid squares and line: untreated control values; solid circles and line: values from rats placed on ATA/ND at time 0; open circles and dashed line: values from rats treated with ATA/ND for 12 weeks and then returned to the normal regimen (Groch and Clifton 1990a).



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