Immunological and Cytogenetic Properties of Developing Thyroid Tumors in the Rat

A. Al Saadi and Gerald J. Mizejewski

Department of Anatomic Pathology, William Beaumont Hospital, Royal Oak, Michigan 48072 [A. A.], and Department of Biology, University of South Carolina, Columbia, South Carolina 29208 [G. J. M.]

SUMMARY

Immunological and cytogenetic changes were studied in iodine deficiency-induced, transplantable “dependent,” “transitional,” and “autonomous” thyroid tumors.

The dependent tumors exhibited minor chromosomal changes and thyroglobulin antigenicity similar to that of normal rat thyroid. The transitional tumors exhibited moderate chromosomal alterations and practically no thyroglobulin antigenicity. The autonomous tumors exhibited severe chromosomal alteration, numerically and/or structurally, and no detectable immunogenicity related to thyroglobulin.

Although progressive cytogenetical and immunological alterations were observed as the tumor “evolved” from dependency to autonomy, no direct relationship has been established between the two.

The results of this study provide criteria to distinguish the dependent tumor from the transitional and autonomous tumors. This is in contrast to previous findings on the iodide peroxidase activity in similar tumors. Iodide peroxidase activity was measurable in dependent and transitional tumors but not in autonomous tumors. Accordingly, it is suggested here that tissue and immunological characteristics are more sensitive to change than are physiological and morphological characteristics in the developing rat thyroid tumors induced by iodine deficiency.

INTRODUCTION

Transplantable thyroid tumors are produced when the thyroid glands of iodine-deficient Fischer rats are implanted s.c. into the flanks of iodine-deficient thyroidectomized rats (5, 14). Earlier, we have reported on the biological behavior as well as the cytogenetic, histopathological, and physiological changes of developing thyroid tumors (1, 2). Accordingly, we have classified such tumors into 3 categories: dependent, transitional, and autonomous. In a more recent investigation, a distinction between the transitional and autonomous tumors was demonstrated, i.e., iodide peroxidase activity was not detectable in the autonomous tumors (15). The sequential immunological changes which might accompany the cytogenetical, biochemical, physiological, and behavioral alterations have not been previously evaluated as these tumors progress from dependency to autonomy. Such information seems to be essential for a more complete characterization and classification of the developing thyroid tumors.

We present here our findings on the antigenic and cytogenetic modulation during the progression of thyroid tumors from dependency to autonomy in the course of serial transplantation.

MATERIALS AND METHODS

Fischer rats bearing transplantable dependent, transitional, and autonomous thyroid tumors were maintained either on a Remington low-iodine diet (containing 0.05 mg of iodine per g of diet) and distilled water or on laboratory chow (containing 2 mg of iodine per g of diet) and tap water.

Tumors

The following iodine deficiency-induced, transplantable thyroid tumors were used. (a) Five Dependent tumors were 1st, 2nd, and 8th generation tumors of papillary and follicular morphology and slow growth rate (2.07 g/month). These tumors grow faster in iodine-deficient, thyroidectomized hosts, (b) Five Transitional tumors were 2nd, 8th, 9th, and 11th generation tumors of follicular morphology with some metastasis to the lung, moderate growth rate (4.8 g/month), and equal growth in iodine-deficient, thyroidectomized and normal control hosts, (c) Four autonomous tumors were 10th through 13th generation tumors of undifferentiated morphology with extensive metastases, rapid growth rate (12.4 g/month), and equal growth in iodine-deficient, thyroidectomized and normal control hosts. (d) Two spontaneous rat mammary tumors and (e) the thyroid glands of 5 normal rats were used as controls.

Cytogenetic Techniques

The procedures used in this study are identical to those previously reported (2). The tumors were aseptically removed, washed, minced, and tissue cultured in McCoy’s 5A Medium (modified) supplemented with 30% fetal calf serum for 2 to 5 days. When a confluent sheet of cells was observed, chromosomal spreads were prepared after Colcemid treatment. At least 50 chromosomal complements were analyzed from each culture.

This work was supported in part by the Marie Williams Memorial Grant P-484 for Cancer Research from the American Cancer Society.

Received August 4, 1971; accepted November 22, 1971.

1 Growth rate of all tumors was measured in both iodine-deficient thyroidectomized and normal control hosts.
Immunological Techniques

Preparation of Antigen and Antisera. The thyroid glands of 35 Fischer rats were homogenized to prepare a 0.85% NaCl extract, following the method of Witebsky and Rose (13). The protein concentration of the antigen extract, determined by Folin's phenol method (4), was 4 mg/ml. Eighty % of the extract was thyroglobulin as determined by cellulose polyacetate electrophoresis, with the use of a known thyroglobulin standard. Purified porcine thyroglobulin served as the glycoprotein standard. Similarly, rat lacrimal gland NaCl extract was prepared for use as a tissue antigen control.

Male New Zealand white rabbits were immunized with 0.25 ml of 1:1 RTE and complete Freund's adjuvant. The rabbits received s.c. biweekly injections of the antigen : adjuvant mixture for 3 weeks. Two weeks following the last injection, the rabbits were bled and the sera were isolated.

Immunodiffusion and IEP. Immunoprecipitation was performed on 1.5% agar plates with double diffusion in 2 dimensions (6). IEP was carried out according to the micromethod of Scheidegger (9).

Fluorescent Antibody. The indirect fluorescent antibody method of Weller and Coons (12) was used throughout this study. Rabbit anti-RTE serum (1 : 4 dilution) was first layered over tumors and normal thyroid frozen sections, incubated for 30 min, and rinsed with phosphate-buffered saline at pH 7.6. The tissue was then layered with goat anti-rabbit immunoglobulin G serum labeled with fluorescein isothiocyanate (1 : 8 dilution). The fluorescein-labeled goat anti-rabbit globulin was absorbed with a mixture of rabbit bone marrow and liver powder prior to use. The slides were incubated and rinsed as above and mounted in phosphate-buffered saline : glycine (1:1) medium. The fluorescent antibody preparations were coded and read in a double-blind fashion with a Leitz Ortholox microscope equipped with a UG-5 exciter filter and a 430-mm barrier filter.

RESULTS

Cytogenetic

Dependent Tumors. All 5 dependent tumors used had a modal number of 42 chromosomes in 48 to 89% of the cells. Eight to 16% of the cells had 41 chromosomes and 4 to 17% had 43 chromosomes. In Tumor 26-733-TX2, 22% of the cells had 40 chromosomes and 9% had 1 or more marker chromosomes, usually a large metacentric and/or a minute chromosome of the small metacentric group which we have earlier characterized as 1 of Pair 15 (Fig. 1B).

Male and Female Tumors. In 4 tumors (3-105-C11, 30-825-C2, 30-979-TX1, 41-805-C9) with 41 chromosomes, 80 to 100% of all the cells had 1 or more marker chromosomes. The number and type of marker chromosomes differed from one tumor to the other; however, large metacentric and submetacentric markers were prevalent (Fig. 1C).

Dependent Tumors. All 5 dependent tumors used had a modal number of 42 chromosomes; however, the percentage of cells with such a chromosome number was lower than that of the dependent tumors (38 to 76%). The primary cytogenetic difference between the dependent and transitional tumors was that the latter had a higher percentage of 41 chromosome cells (18 to 35%). The majority (80 to 95%) of the cells with 41 chromosomes were found to have a karyotype missing 1 chromosome of the small metacentric group which we have earlier characterized as 1 of Pair 15 (Fig. 1B).

Autonomous Tumors. Usually, the autonomous tumors have a numerically and/or structurally altered karyotype. Although the modal number of 3 of the 4 studied tumors was still 42 chromosomes, 80 to 100% of all the cells had 1 or more marker chromosomes. The number and type of marker chromosomes differed from one tumor to the other; however, large metacentric and submetacentric markers were prevalent (Fig. 1C).

Sereology. Immunofluorescence revealed the presence of 2 distinct bands when rabbit anti-RTE serum was tested against RTE antigens (Fig. 2). This finding is compatible with results obtained by others on human thyroid (7). No cross-reaction was observed when lacrimal gland extract was tested against anti-RTE serum. Two bands also were obtained when the cellulose polyacetate electrophoresis technique was used. Immunoelectrophoretic analysis demonstrated that the antibodies directed against the RTE antigen were immunoglobulin (IgG), (Fig. 3A). When RTE antigen was subjected to electrophoresis and tested against rabbit

Table I

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Animal and tumor identification</th>
<th>Fluorescent antibody grading&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent</td>
<td>30-123-C8</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>30-821-C2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>26-718-TX1</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>34-716-TX1</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>26-733-TX2</td>
<td>+</td>
</tr>
<tr>
<td>Transitional</td>
<td>30-301-C8</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>30-979-TX8</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>41-805-C9</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>30-825-C2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>53-110-C11</td>
<td>–</td>
</tr>
<tr>
<td>Autonomous</td>
<td>25-227-C11</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>40-980-TX12</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>50-978-TX13</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>25-734-C10</td>
<td>–</td>
</tr>
<tr>
<td>Mammary</td>
<td>20-113-TX11</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>20-299-C11</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup> +, faint colloidal staining and positive epithelial cell fluorescence; +, positive colloidal and epithelial cell fluorescence; –, absence of specific immunofluorescence.
Antiserum, 2 bands were detected (Fig. 3B). The α-2-band corresponded to a known porcine thyroglobulin standard.

**Immunofluorescence.** The normal rat thyroid gland exhibited the classical fluorescent staining pattern described by others (3). In brief, these patterns consisted of a bright, apple-green fluorescence in the colloid and apical regions of the follicular cells. A dull green fluorescence was observed in the blood vessels, which probably represented nonspecific IgG staining. The background appeared blue-green.

Table 1 summarizes the immunofluorescent results of all of the tumors used in this study.

All 5 dependent tumors exhibited positive fluorescent staining either in the colloid, the cells, or both (Fig. 4). The intensity of fluorescent staining of the cells of all tumors was similar to that of normal thyroid cells; however, the staining intensity in the colloid showed some marked variation. The staining intensity of the colloid of 2 tumors (26-718-TX1 and 30-821-C2) was similar to or approached that of normal thyroid. The remaining 3 tumors showed less intense fluorescent staining in the colloid. No specific fluorescence was observed in the autonomous tumors. Although all transitional tumors had some follicular configuration, no detectable fluorescent reaction was observed in the follicular lumen. The epithelial cells of 1 transitional tumor (Table 1) displayed faint, positive, fluorescent staining.

**DISCUSSION**

The classification of iodine deficiency-induced thyroid tumors in the rat into dependent, transitional, and autonomous was originally based on biological behavior, morphology, and cytogenetics. More recently, a distinction between the autonomous tumors on one hand and the dependent and the transitional tumors on the other was established (15). Iodide peroxidase activity was measurable in both dependent and transitional tumors but not in autonomous tumors.

The results of this study provide criteria to distinguish the dependent tumor from the transitional and autonomous tumors. As a tumor progresses from dependency to autonomy, specific immunological changes seem to occur. The positive immunofluorescent staining of the dependent tumor displayed antigenic similarities to that of the normal rat thyroid. The lumen as well as the follicular cells of the dependent tumors showed classical thyroid fluorescent staining patterns. This suggests that the molecular configuration of thyroglobulin has not undergone major structural alteration. The secondary and tertiary molecular arrangements that provide the immunological determinants of thyroglobulin appear unchanged. However, finer changes in the molecular configuration could have occurred but, if so, were beyond the sensitivity of the technique used. No specific immunofluorescent staining was detected in any of the autonomous tumors. Generally, the transitional tumors were similar to the autonomous tumors, except for occasional fluorescent staining in the cytoplasm of the epithelial cells. Although microfollicular configuration was the predominant histological characteristic of the transitional tumors, immunofluorescence was not observed in the lumen (colloid?) of the microfollicle. This observation is compatible with the results of Salabe and Robbins (8) on rat thyroid tumors similarly induced. Their results indicate that thyroglobulin in microfollicular tumors was present with the ribosomal but not the soluble fraction. Similar results were also reported on human thyroid tumors (10, 11).

The presence or absence of immunogenicity of thyroid tumors appeared to be related to the functional state and the cytogenetic composition of these tumors. The autonomous tumors, which had lost their ability to concentrate iodine and synthesize iodinated compounds and which exhibited severe chromosomal changes, had either lost, or undergone alteration in, their immunogenic characteristics. The dependent tumors, which were capable of concentrating iodine as well as synthesizing iodinated compounds, exhibited minor chromosomal changes and were found to retain their immunogenic characteristics related to thyroglobulin. The transitional tumors were functional, exhibited moderate chromosomal abnormalities, and displayed little or no normal thyroglobulin antigenicity.

The progression of transplantable rat thyroid tumor from dependency to autonomy seems to be associated with complete or partial loss of function and antigenecity as well as increased chromosomal abnormalities. No distinction between dependent and transitional tumors could be made on the basis of the iodide peroxidase activity. In contrast, such distinction seems to be possible on the basis of the immunological results we report here. Accordingly, it seems that the loss or alteration of antigenic characteristics of thyroid tumors precedes certain enzymatic deletions. Iodide peroxidase activity was detectable in both dependent and transitional tumors but not in autonomous tumors. However, only the dependent tumors retained immunological characteristics similar to those of normal thyroid tissue. Transplantation antigens of these tumors were not studied, and such antigens may continue to be present after the specific thyroid immunogenicity is lost.

It could be concluded, therefore, that functional and morphological specificities are retained beyond the immunological specificity in the thyroid tumors. Alterations of tissue-specific antigens could lead to or accompany morphological and functional changes in the thyroid tissue. It is tempting to interpret the sequential changes in the transplanted thyroid tumors as they "progress" from dependency to autonomy on the basis of gradual loss of tissue-specific traits. Some of the tissue immunological characteristics appear to be altered earlier than are the tissue functional and morphological characteristics. Thyroid tumor cells seem to retain their morphological functional identity far beyond their immunological identity.

Although no direct relationship between the chromosomal and the immunogenic alterations could be directly deduced from these results, it is obvious that the 2 phenomena occur together and progress in the same direction. It is rather difficult to establish a cause-effect relationship between the chromosomal and immunogenic changes; however, it is strongly felt the chromosomal changes precede the observed immunogenic changes.
REFERENCES


Fig. 1. Representative karyotypes of the 3 categories of tumors: A, karyotype of a dependent tumor. Arrow 2, a large metacentric marker chromosome; Arrow 1, a missing chromosome. B, typical karyotype of a 41-chromosome cell from a transitional tumor. Eighteen to 35% of the transitional tumor cells were 41-chromosome cells, each missing a small metacentric chromosome (No. 15?; arrow). C, the autonomous tumor, exhibiting a severely altered karyotype. Arrow 1, the missing chromosome; Arrow 2, 3 metacentric marker chromosomes.
Thyroid Tumor Immunology

If 11, C3C nodilfusion revealed the presence of 2 distinct bands. Wells A to E contain increasing 2-fold dilutions of RTE. Well F contains rabbit anti-RTE serum.

RTE antigen was placed in the trough. Wells A to E were observed when RTE antigen was subjected to electrophoresis (well) and tested against rabbit anti-RTE serum.

In B, two bands were observed when RTE antigen was subjected to electrophoresis and tested against rabbit anti-RTE serum. Staining occurred in both the colloid and epithelial cells in the thyroid-dependent tumors. Oil, X 450.

Fig. 2. Ouchterlony immunodiffusion revealed the presence of 2 distinct bands. Wells A to E contain increasing 2-fold dilutions of RTE. Well F contains rabbit anti-RTE serum.

Fig. 3. In A, the antibodies produced against the RTE antigen were IgG as determined by IEP. Rabbit anti-RTE serum was placed in the wells.

Fig. 4. Positive fluorescent staining occurred in both the colloid and epithelial cells in the thyroid-dependent tumors. Oil, X 450.

Fig. 5. Positive immunofluorescence was not present in the transitional tumors with the exception of occasional epithelial cell fluorescence. Oil, X 450.
Immunological and Cytogenetic Properties of Developing Thyroid Tumors in the Rat

A. Al Saadi and Gerald J. Mizejewski


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/32/3/501

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.