

# THYROID TODAY<sup>®</sup>

Published Quarterly

Editor: J.H. Oppenheimer, MD  
Volume VIII, Number 4  
October/November/December 1985

ISSN 0190-0625

## IMMUNOGLOBULINS AFFECTING THYROID GROWTH



Hemmo A. Drexhage, MD  
Senior Lecturer in Clinical Immunology  
Department of Pathology  
Free University Hospital  
Amsterdam  
The Netherlands

It is ironic that the most prevalent and most complicated form of thyroid disease bears the name of "simple goiter." The synonyms and variants include euthyroid, nontoxic, diffuse, or multinodular goiters and toxic nodular goiters. Most of these goiters are endemic and mainly due to iodine deficiency or goitrogens in the diet or water supply. However, even in areas where none of these environmental influences apply, the prevalence of euthyroid goiter still ranges from 1% to 5%.

Studer<sup>1</sup> has given the following definition of simple goiter: "Simple goiter is a slowly developing diffuse or nodular enlargement of the thyroid gland resulting from excessive replication of epithelial cells with subsequent generation of new follicles of widely differing structure and function." Whereas in Graves' disease the individual activities of thyroid acini are tightly coordinated with each other, such coordination does not exist in simple goiters. As a consequence there are wide differences in function between one follicle and the next, and differences in their sizes, thyroglobulin content, and other biochemical parameters.

Although thyroid autoimmunity has been studied intensively since 1956, the possibility that simple goiters may also arise from this process was raised only in the past ten years.<sup>2,3</sup> The conventionally recognized triad of primary autoimmune thyroid diseases (Graves' disease, Hashimoto's disease, and primary myxedema) characteristically exhibit increased values of thyroglobulin or thyroid microsomal antibodies in patients' sera. Patients with sporadic simple goiter do not show such distinctive increases, though trace levels are found with the sensitive hemagglutination tests in approximately 30% to 40% of such patients, or about twice the expected frequency of normal female controls. Moreover, using an ultrasensitive assay for immunoglobulins competing with thyroid-stimulating hormone (TSH) for its receptor (TBII, thyrotropin-binding inhibiting immunoglobulin), Brown et al<sup>3</sup> were able to demonstrate that a large proportion of

simple goiters gave weak positive responses. Further, histologically, simple goiters occasionally show small areas of lymphocytic infiltrations. Although these observations were suggestive, it was not until 1980 that the existence of immunoglobulins that stimulate thyroid growth (TGIs, thyroid-growth-stimulating immunoglobulins) was evident in patients with simple goiters.<sup>4</sup>

### Cytochemical Bioassays for TGI

Bitensky et al<sup>5</sup> developed the initial cytochemical bioassay for thyroid-stimulating immunoglobulins (TSIs) and this test probably still remains the most sensitive method available. It is based on the labilization of thyroid lysosomes by TSH or by TSI. This is one of the rapid effects of TSH and one that is related to hormone biosynthesis and secretion. To assay the effects of various agents on cell growth, which involves the slower actions of TSH, we focused on a different cellular event: the synthesis of DNA.<sup>4</sup> Segments of guinea pig thyroid glands were maintained for five hours in the presence of immunoglobulins obtained from patients' sera. DNA synthesis was assessed by measuring the increase in DNA per nucleus in sections from the thyroid segments as stained by the Feulgen reaction. TSH standards were included as a positive control, whereas negative controls consisted of immunoglobulins from normal subjects. Results of growth are expressed as the percentage of cells in the S-phase, that phase of the cell cycle characterized by active DNA synthesis (Fig 1a).

The optimal concentration of immunoglobulin for the Feulgen cytochemical bioassay (CBA) was defined by dose-response curves, using immunoglobulins obtained from patients with simple goiters and untreated thyrotoxic Graves' disease patients with large goiters. The dose response data of these immunoglobulins were compared with those of the human TSH standard. In all instances bell-shaped curves were observed (Fig 1), comparable to those obtained in the cytochemical bioassay for TSI based on lysosomal labilization. The optimal amount for the TSH standard ranged from 0.01 to 1.0  $\mu$ U/ml culture fluid. For immunoglobulins from patients with untreated goitrous Graves' disease, the optimal doses ranged from 15  $\mu$ g to 125  $\mu$ g, but in simple goiter a higher concentration of immunoglobulin was needed to reach the top of the bell-shaped curve, ie, 125 to 500  $\mu$ g immunoglobulin/ml culture medium (Fig 1b). This shows that TGI from Graves' disease is approximately tenfold more potent in inducing growth in the guinea pig thyroid as compared to that obtained from simple goiter.

and although the thymidine incorporation method is technically simpler, its lesser sensitivity limits general application. These considerations appear also to apply to efforts to study thymidine incorporation into the nuclei of cells of a continuously replicating rat thyroid cell line code-named FRTL-5. Valente et al<sup>9</sup> showed that 17 of 20 patients with active Graves' disease and two of five patients with Hashimoto's goiter yielded immunoglobulin preparations that augmented cell growth in this system. These investigators, however, could not detect any growth-promoting antibodies in simple goiter occurring in the New England area, nor did they find any correlation between goiter size and *in vitro* TGI activity.

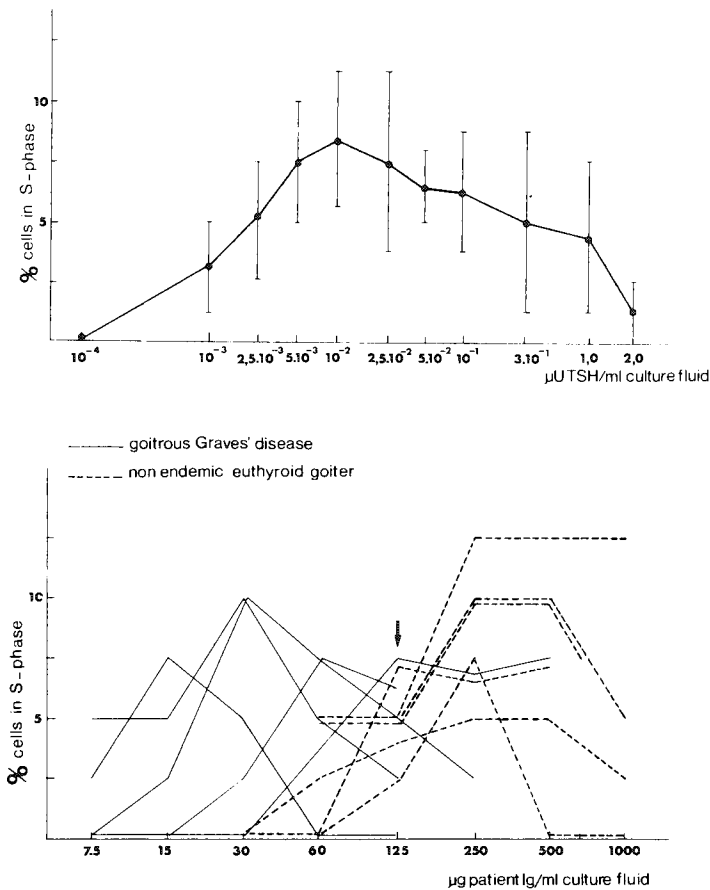
### TGI and the Thyrotoxicosis of Graves' Disease

Most patients with Graves' disease have some enlargement of their thyroid gland, but the size of the goiter bears little relationship to the amount of T<sub>3</sub> or thyroxine (T<sub>4</sub>) generated. Moreover, about 2% to 10% of thyrotoxic patients have no goiter. In our initial studies, we established a general relationship between the size of the thyrotoxic goiter as estimated by palpation or thyroidectomy and the intensity of the growth-promoting stimulus elicited by the immunoglobulin preparation. In patients with thyroid glands that were either not palpable or barely palpable but with significant increases of circulating hormones, the effects of immunoglobulins were indistinguishable from those of immunoglobulins obtained from normal subjects. There were no correlations between the pretreatment T<sub>3</sub> level and the immunoglobulin response. These data have now been confirmed by several groups.<sup>7,8,9</sup>

It is thus clear that several distinctive classes of immunoglobulins participate in the pathogenesis of Graves' disease. TSI stimulates thyroid follicle cells to produce excessive amounts of T<sub>3</sub> and T<sub>4</sub>, whereas TGI stimulates follicle cells to grow, independent of the action of TSI.

There is also evidence that TSI and TGI react with different domains of the TSH receptor. Valente et al<sup>10</sup> generated human monoclonal antibodies from heterohybridomas obtained by fusing mouse myeloma cells with peripheral lymphocytes from patients with active Graves' disease. Four antibodies were characterized as presumptive thyrotropin receptor antibodies, since they specifically inhibited thyrotropin binding. Two of these antibodies were representative of autoimmune stimulators in Graves' disease, since they could stimulate thyroid function in several assays, including the mouse bioassay. These stimulating antibodies interacted strongly with human thyroid ganglioside preparations, but were poor inhibitors of <sup>125</sup>I-thyrotropin binding. One of the monoclonal antibodies stimulated thymidine incorporation in the FRTL-5 cell line and also showed positive results in the Feulgen cytochemical bioassay. This monoclonal TGI had no intrinsic stimulatory action in assays of thyroid function but rather inhibited thyrotropin activity in the assays. Although this antibody did not react with human thyroid gangliosides it inhibited the binding of TSH to its receptor. This indicates that TGI may interact with groupings on the TSH receptor differently from those reacting with TSI.

These data emphasize the multiplicity of antigenic sites with which the immunoglobulins of Graves' disease react. The autoimmune B-cell response in Graves' disease can be considered as polyclonal, involving the production of several TSIs and TGIs, TSH receptor-blocking antibodies, as well as variable amounts of antimicrobial and antithyroglobulin antibodies. The diversity of biologically active antibodies may well account for the variation in clinical manifestations.



**Fig 1.** Dose-response curves for thyroid growth obtained in Feulgen CBA with: (a) human TSH (Medical Research Council-A standard nr 63-14), showing mean values (n=15) and (b) immunoglobulin preparations from patients with sporadic goiter (broken lines) and cases of goitrous Graves' disease (solid lines).

TGI can also be assayed in the organ culture system with another quantitative cytochemical method that measures activity of glucose-6-phosphate dehydrogenase (G6PD).<sup>6</sup> With this method McMullan and Smyth<sup>7</sup> recently found TGI to be present in all 17 goitrous Graves' disease patients tested, and also in some 70% of patients with simple goiter. Four out of eight with toxic nodular goiters were positive for TGI as well. Recently we obtained similar data in toxic nodular goiter, showing 7 of 9 patients TGI-positive in the Feulgen densitometric assay (Wiener and van der Gaag, in preparation).

Efforts have also been directed at developing other methods for measuring TGI levels. Chiovato et al<sup>8</sup> have tested the effect of immunoglobulins on rat thyroid follicles separated by collagenase treatment and subsequently cultured. Fifteen of 22 preparations obtained from patients with goitrous Graves' disease (68%) showed an increase in 3H-thymidine incorporation in the DNA of thyroid cells, an indication of a growth-promoting effect. However, the immunoglobulins from 15 thyrotoxic patients without apparent glandular enlargement did not differ from normal immunoglobulin. Among the preparations that yielded a response, there appeared to be a correlation between the activity of the immunoglobulin and the size of the gland, but not with the level of circulating triiodothyronine (T<sub>3</sub>). Among patients with simple goiters, three of nine patients with recurrences after thyroidectomy and one patient with familial nodular goiter yielded positive results. The strongest TGI response from a recurrent nontoxic goiter patient was 40 times less potent than that from a patient with Graves' disease. Although in principle these results are similar to those obtained with the cytochemical bioassay procedure,



lymphocytic thyroiditis (primary myxedema) may be due to the ability of the follicular cells of Hashimoto's goiters to regenerate in response to increased pituitary TSH secretion. In primary myxedema this repair mechanism may be blocked immunologically. Blocking antibodies have not been demonstrated in goitrous Hashimoto's disease. On the contrary, TGIs were detectable in approximately half of the patients, mainly in those where the goiter could not be reduced with T<sub>4</sub> therapy or those showing a postoperative recurrence. The TGI in these patients may augment the effect of TSH in the repair of follicle cells, thus contributing to the goiter formation<sup>17</sup> (Fig 3). Genetic differences between goitrous Hashimoto's disease and atrophic lymphocytic thyroiditis have also been found<sup>18</sup>: an increased prevalence of HLA-DR3 in primary myxedema patients, whereas goitrous Hashimoto's thyroiditis patients show a predominance of HLA-DR5.

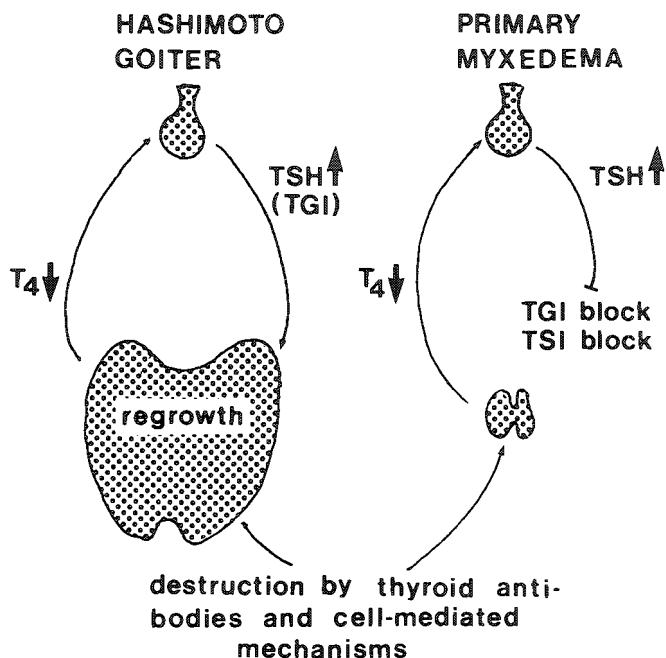


Fig 3. Both Hashimoto goiter and primary myxedema are due to destructive lymphoid invasion of the gland, counteracted by constant generation of new acini under the influence of TSH (and some additional TGI) in Hashimoto goiter. In primary myxedema, the repair mechanism is blocked by TGI block and TSI block, which ultimately leads to atrophy of the gland.

Mothers with thyroid atrophy occasionally give birth to babies with a familial form of congenital athyreotic cretinism. Goldsmith et al<sup>19</sup> were the first to study such a family in detail. Thyroid suppression was present in all six offspring of a myxedematous mother showing positive thyroid microsomal antibodies. Two of the siblings died in the neonatal period. Although hypothyroidism was transient in two of the remaining offspring, all were mentally and physically retarded at a later age, despite full T<sub>4</sub> replacement therapy. To account for the familial clustering of this form of cretinism the authors postulated the transplacental passage of a "thyrocytotoxic" factor in addition to thyroid microsomal antibodies.

More recently, Japanese investigators provided the evidence for the transplacental passage of a TBII capable of blocking TSH-induced adenylate cyclase stimulation and interfering with thyroid hormone synthesis.<sup>13,14</sup> The maternal antibody was present in high titer in the siblings at 2 months of age. The levels decreased at 3 months and tests were negative at approximately 10 months of age. These children had only mild transient forms of congenital hypothyroidism. We have studied a Turkish family in which four members (two sisters, an aunt, and an uncle) were affected by

more persistent forms of congenital hypothyroidism. In three, the thyroid gland could not be seen by radioactive thyroid imaging. The mother of the two sisters was clinically euthyroid throughout the period of observation, but showed high levels of microsomal antibodies in addition to immunoglobulins that blocked the trophic

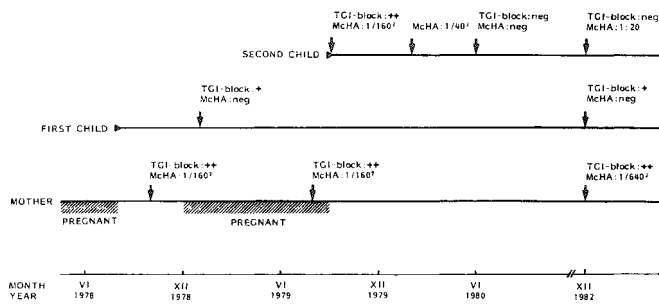


Fig 4. Thyroid microsomal antibodies (McHA) and TGI block in a euthyroid mother, who gave birth to two girls with a familial form of congenital hypothyroidism. The first child produced the thyroid antibodies herself; in the second child there is proof of the transplacental passage of such antibodies.

action of TSH. The serum of the father was weakly positive for microsomal antibodies. The maternal growth-blocking antibodies underwent transplacental transfer in the younger child. The older sibling, the aunt, and the uncle produced growth-blocking antibodies themselves and may thus represent forms of thyroid autoimmunity with a very early onset (Fig 4). These family studies indicate that familial forms of congenital hypothyroidism are complex disorders and, at least in some cases, may be brought about by transplacental passage of thyroid receptor antibodies, whereas others may be associated with the inheritance of a trait for thyroid autoimmunity.

Intriguing are recent data on TGI block in sporadic forms of congenital hypothyroidism.<sup>20</sup> This disorder has a prevalence of about 1 to 4,000 live births in iodine-replete areas (see *Thyroid Today*, Volume 8, Number 1, Jan/Feb/March 1985). Most industrialized countries now screen all newborns for the presence of this disease in an attempt to eradicate the permanent neurological sequelae of delayed T<sub>4</sub> replacement therapy.<sup>21</sup> The pathogenesis of sporadic congenital hypothyroidism is largely unknown.

Of 34 mothers of infants detected in the Quebec screening program (1979 to 1983), 15 serum samples were positive for TGI block when tested in the ultrasensitive Feulgen cytochemical bioassay. At childbirth, all mothers were clinically and biochemically euthyroid. In general, the blocking immunoglobulins were found in the absence of thyroid microsomal antibodies. Two mothers, however, had significant titers of antimicrosomal antibodies and thus had an autoantibody profile similar to that which characterizes adult thyroid atrophy. They were the only two mothers who became hypothyroid in a follow-up of one to three years, and their infants can be considered to be affected by familial congenital hypothyroidism. Sixteen postpartum infant blood samples from the Quebec program were also available for study; eight were positive for immunoglobulins that blocked TSH-induced thyroid growth. There was a generally good correlation in the positivity for these immunoglobulins between mothers and their own children.

Seven mothers with positive sera were tested up to three years after childbirth, when the results for four of the seven were negative. This suggests that blocking immunoglobulins may disappear from the maternal circulation in the course of time.

The data from the Quebec series raise the possibility that a transplacental passage of maternal immunoglobulins influencing

TSH-induced processes of thyroid growth may play a role in the pathogenesis of a substantial proportion of cases of sporadic congenital hypothyroidism. It is not clear, however, whether these antibodies arise as a result of a true maternal thyroid autoimmune process or whether their formation is induced by some sort of viral infection of the thyroid or by the fetal-placental unit. The latter is conceivable in view of the fact that the placenta is a major source of many polypeptide hormones, some of which have thyrotrophic action, such as chorionic gonadotropin. Maternal antibodies to such placental thyrotrophic hormones, to their corresponding idiotypes,<sup>22</sup> or to placental receptors for such hormones could crossreact with receptors present on fetal thyroid cells. These considerations are, however, highly speculative and need further evaluation.

### Future Developments

It is clear from the data summarized here that TGI and TGI block play an important role in functional and morphological disturbances of the thyroid in a broad spectrum of thyroid disorders, including primary myxedema, Graves' disease, simple sporadic goiter, Hashimoto's disease, and congenital hypothyroidism. The assay of immunoglobulins affecting the biological activity of the thyroid gland will probably become increasingly important in clinical practice. Development of simplified methods to avoid the laborious techniques currently in use would clearly be advantageous.

Recent reports have suggested that patients with endemic goiters may also have growth-promoting antibodies; 50% to 60% of nontoxic simple goiter cases from Tuscany and Brazil<sup>23,24</sup> showed values positive for TGI in the FRTL-5 cell assay. Iodized oil treatment resulted in a disappearance of TGI from the circulation of almost every patient initially positive. These findings highlight what appears to be a potentially fascinating relationship between intrathyroidal metabolism and the autoimmune response and point to the need for further clarification.

Continuous glandular TSH stimulation in animals results first in diffuse, but later in nodular, goiter development. In these nodular goiters areas of malignancy may eventually occur.<sup>25</sup> These considerations prompt a systematic study of TGI levels in patients with thyroid carcinoma.

Another avenue of exploration is opened by the newer concepts of the factors responsible for the growth of endocrine cells. Diacylglycerol and inositolpolyphosphate have been proposed as second messengers. Both play a role in the phosphatidyl-inositol turnover pathway, which is important in cell proliferation.<sup>26,27</sup> This pathway can be triggered by stimulating the TSH receptor, and also by stimulating other receptors such as those for epidermal growth factor (EGF), adrenaline, and lectins. Thus, immunoglobulins affecting thyroid growth may not necessarily be directed to the TSH receptor. They may equally be specific for other known and unknown receptors. If so, this would provide an explanation for the lack of strong overlap between Graves' disease and simple goiter. Future research will unravel whether there are several classes of TGI specific for different receptors instrumental in guiding cell proliferation.

### References:

1. Studer H: A fresh look at an old thyroid disease: Euthyroid and hyperthyroid nodular goitre. *J Endocrinol Invest* 1982;5:57-63.
2. Doniach D, Marschall NJ: Autoantibodies to the thyrotrophin receptors on thyroid epithelium and other tissues, in Talal N (ed): *Autoimmunity*. New York, Academic Press, 1977, pp 621-642.
3. Brown RS, Pohl SL, Jackson IMD, et al: Do thyroid stimulating immunoglobulins cause nontoxic and toxic nodular goitre? *Lancet* 1978;1:904-906.
4. Drexhage HA, Bottazzo GF, Doniach D, et al: Evidence for thyroid-growth-stimulating immunoglobulins in some goitrous thyroid diseases. *Lancet* 1980;2:287-292.
5. Bitensky L, Alaghband-Zadeh J, Chayen J: Studies on thyroid stimulating hormone and the long-acting thyroid stimulating immunoglobulin. *Clin Endocrinol* 1974;3:363-374.

6. Drexhage HA, Hammond LJ, Bitensky L, et al: The involvement of the pentose shunt in thyroid metabolism after stimulation with TSH or with immunoglobulins from patients with thyroid disease. *Clin Endocrinol* 1982;16:49-63.
7. McMullan NM, Smyth PPA: In vitro generation of NADPH as an index of thyroid stimulating immunoglobulins (TGI) in goitrous disease. *Clin Endocrinol* 1984;20:269-280.
8. Chiovato L, Hammond LJ, Hanafusa T, et al: Detection of thyroid growth immunoglobulins (TGI) by 3H-thymidine incorporation in cultured rat thyroid follicles. *Clin Endocrinol* 1983;19:581-590.
9. Valente WA, Vitti P, Rotella CM, et al: Antibodies that promote thyroid growth: A distinct population of thyroid-stimulating autoantibodies. *N Engl J Med* 1983;309:1028-1034.
10. Valente WA, Vitti P, Yavin E, et al: Monoclonal antibodies to the thyrotropin receptor, stimulating and blocking antibodies derived from the lymphocytes of patients with Graves' disease. *Proc Natl Acad Sci USA* 1982;79:6680-6684.
11. Strakosch CR, Wenzel BE, Row VV, et al: Immunology of autoimmune thyroid diseases. *N Engl J Med* 1982;307:1499-1507.
12. Orgiazzi J, Williams DE, Chopra IJ, et al: Human thyroid adenyl-cyclase stimulating activity in immunoglobulin G of patients with Graves' disease. *J Clin Endocrinol Metab* 1976;42:778-781.
13. Matsuura N, Yamada Y, Nohara Y, et al: Familial neonatal transient hypothyroidism due to maternal TSH-binding inhibitor immunoglobulins. *N Engl J Med* 1980;303:738-741.
14. Iseki M, Shimizu M, Oikawa T, et al: Sequential serum measurements of thyrotropin binding inhibitor immunoglobulin G in transient familial neonatal hypothyroidism. *J Clin Endocrinol Metab* 1983;57:384-389.
15. Steel NR, Weightman DR, Taylor JJ, et al: Blocking activity to action of thyroid stimulating hormone in serum from patients with primary hypothyroidism. *Br Med J* 1984;288:1559-1562.
16. Drexhage HA, Bottazzo GF, Bitensky L, et al: Thyroid growth-blocking antibodies in primary myxoedema. *Nature* 1981;289:594-596.
17. Doniach D: Hashimoto's thyroiditis and primary myxoedema viewed as separate entities. *Eur J Clin Invest* 1981;11:245-247.
18. Walfish PG, Farid NR: The immunogenetic basis of autoimmune thyroid disease, in Walfish PG, Wall JR, Volpe R (eds): *Autoimmunity and the Thyroid*. Orlando, Academic Press, 1985, pp 9-36.
19. Goldsmith RE, McAdams AJ, Larsen PR, et al: Familial autoimmune thyroiditis: Maternal fetal relationship and the role of generalized autoimmunity. *J Clin Endocrinol Metab* 1973;37:265-275.
20. Van der Gaag RD, Drexhage HA, Dussault JH: Role of maternal immunoglobulins blocking TSH-induced thyroid growth in sporadic forms of congenital hypothyroidism. *Lancet* 1985;1:246-250.
21. Dussault JH, Walker P: *Congenital Hypothyroidism*. New York, Marcel Dekker, 1983.
22. Roitt IM, Male DK, Cooke A, et al: Idiotypes and autoimmunity. *Springer Semin Immunopathol* 1983;6:51-66.
23. Kohn LD, Valente WA, Alvarez FV, et al: New procedures for detecting Graves' immunoglobulins, in Walfish PG, Wall JR, Volpe R (eds): *Autoimmunity and the Thyroid*. Orlando, Academic Press, 1985, pp 217-248.
24. Schatz H, Beckmann FH, Floren M: Radioassay for thyroid growth stimulating immunoglobulins (TGI) with cultivated porcine thyroid follicles. *Horm Metab Res* 1983;15:627-628.
25. Williams ED: Hyperplasia and neoplasia in endocrine glands, in Williams ED (ed): *Current Endocrine Concepts*. Eastbourne, East Sussex, England, Praeger Publishers, 1982, pp 3-10.
26. Marx J: A new view of receptor action. *Science* 1984;224:271-274.
27. Van Herle AJ, Vassart G, Dumont JE: Control of thyroglobulin synthesis and secretion. *N Engl J Med* 1979;301:239-249.

### Glossary of Abbreviations

- cAMP**—cyclic adenosine monophosphate  
**CBA**—cytochemical bioassay  
**DNA**—deoxyribonucleic acid  
**EGF**—epidermal growth factor  
**FRTL-5**—continuously replicating rat thyroid cell line  
**G6PD**—glucose-6-phosphate dehydrogenase  
**IgG**—immunoglobulin G  
**MCHA**—thyroid microsomal antibodies  
**TBII**—thyrotropin-binding inhibiting immunoglobulin  
**TGI**—thyroid-growth-stimulating immunoglobulin  
**TSH**—thyroid-stimulating hormone  
**TSI**—thyroid-stimulating immunoglobulin  
**T<sub>3</sub>**—triiodothyronine  
**T<sub>4</sub>**—thyroxine