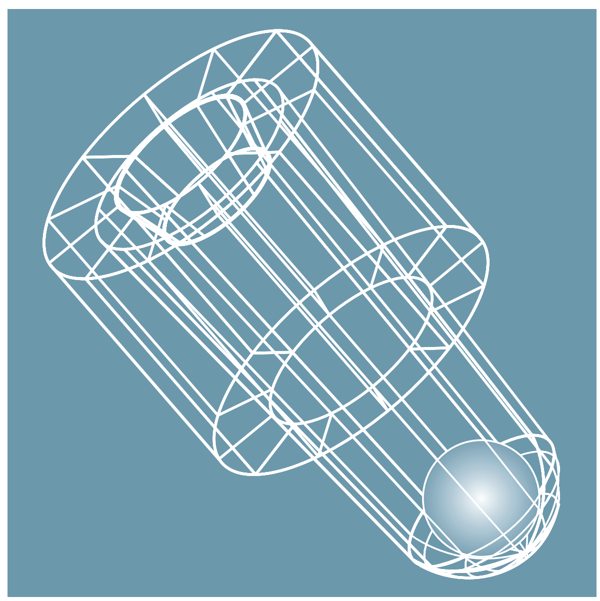
DPC Technical Report

IMMULITE®

Thyroglobulin and Antithyroglobulin Autoantibodies in Differentiated Thyroid Cancer



Peter Bodlaender, Ph.D.
Technical Director
Diagnostic Products Corporation

DPC®

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Thyroglobulin

Pathophysiology

The thyroid gland synthesizes, stores and releases thyroid hormones—thyroxine (T4) and triiodothyronine (T3)—into the circulation as required. The gland consists of clusters of thyroid follicles (acinar units) which are constructed with a single layer of cuboidal epithelial cells surrounding a cavity (lumen). This cavity is filled with colloid containing iodinated thyroglobulin molecules, the storage form of thyroid hormones. (See Figure 1.)

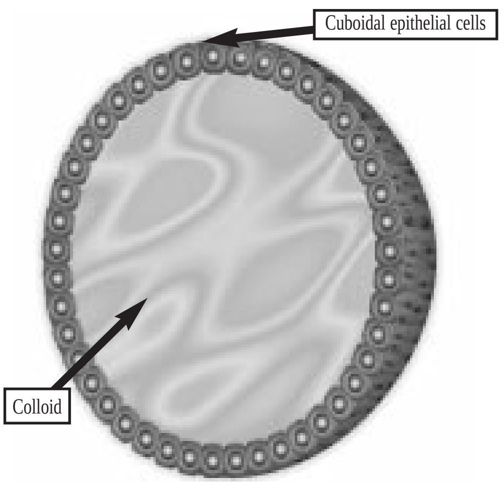

Figure 1. Thyroid follicle (acinar unit) filled with colloid containing thyroglobulin.

The initial step in the biochemical pathway leading to the production of thyroid hormones (Figure 2) involves active transport of iodine against a steep concentration gradient. Production of thyroglobulin (Tg), a large molecular weight glycoprotein of approximately 660 kD, is a specialized function of thyroid cells. Tg serves as the protein framework within which thyroid hormones are synthesized and stored. Tyrosyl residues, abundant in Tg, are enzymatically iodinated to produce monoiodo- and diiodotyrosines. Adjacent iodinated tyrosines, appropriately oriented to each other, are then enzymatically coupled to produce T4 and T3 residues which remain as part of the thyroglobulin amino acid chain. The chemically modified Tg molecules are extruded as colloid into the lumen.

Release of thyroid hormones first involves reentry (endocytosis) of a small droplet of colloid into the follicular cell. There, the droplet is fused with lysosomes (vacuoles containing proteolytic enzymes), and the Tg molecules are hydrolyzed into their constituent amino acids. The preformed thyroid hormones are then liberated and released into the circulation.

Tg is not completely secluded within the thyroid follicle, however. A small fraction normally escapes into the circulation by an unidentified process. Factors that influence serum Tg levels include:

1. Mass of differentiated thyroid tissue. Tg levels are not detected in the complete absence of differentiated thyroid tissue.
2. Physical damage or inflammation of the thyroid. Disruption of the architectural integrity of the thyroid follicle, as by differentiated thyroid cancer, can cause large amounts of Tg to leak into the circulation.
3. Thyrotropin receptor stimulation. In Graves' disease, thyroid receptor autoantibodies stimulate the thyroid gland to produce and release excessive amounts of thyroid hormones (hyperthyroidism) as well as higher than normal amounts of Tg into the circulation.[7,9]

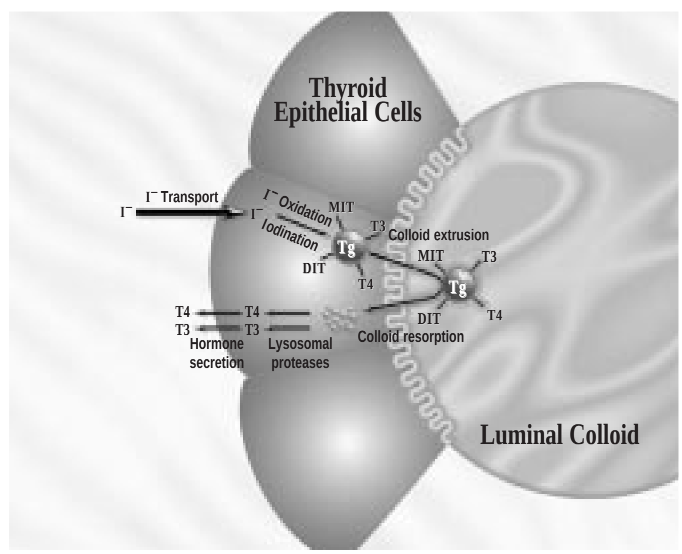

Figure 2. Role of thyroglobulin (Tg) in thyroid hormone production. Intracellular iodination of Tg leads to the production of thyroid hormone (T4 and T3) residues within the molecule, which are then stored in the luminal colloid. When T4 and T3 are needed, Tg gains entry into the cell via endocytosis. T4 and T3 are enzymatically released from the Tg molecule and secreted into the circulation. MIT = monoiodotyrosine; DIT = diiodotyrosine.

Clinical Utility of Tg Determinations

The thyroid tissue-specific origin of Tg makes it a useful marker for functioning, differentiated thyroid tissue, and it can serve as an independent measure of the degree of TSH suppression. The absence of Tg provides strong evidence for the lack of functioning thyroid tissue, and measurements may be helpful for the differential diagnosis of neonatal congenital hypothyroidism.[5] Tg determinations may also be helpful in distinguishing between thyrotoxicosis factitia and subacute thyroiditis. Both conditions are associated with elevated thyroid hormone levels, but the inflammation of thyroiditis is associated with greater than normal leakage of Tg into the circulation. In contrast, exogenous administration of thyroid hormones suppresses circulating Tg levels into the low-normal or nondetectable range.[5]

The major clinical value of Tg measurements, however, is for managing, not diagnosing, differentiated thyroid cancer. Tg levels may be increased in a variety of benign conditions as well as in differentiated thyroid cancer; elevated Tg levels do not distinguish between them. Serial determinations over time, however, can serve as an extremely sensitive adjunct for the management of differentiated thyroid cancer. In patients who have had total thyroid ablation, a good prognosis is indicated by a decrease in Tg concentrations to undetectable levels, and such a course identifies low-risk patients. Postoperative elevation of Tg, particularly during suppressive therapy, provides a very sensitive indication of residual local or metastatic cancer and identifies high-risk patients.

Use of Tg measurements to identify low-risk patients reduces reliance on whole-body radioiodine scans, which are invasive and far more expensive. Such imaging techniques could be reserved primarily for the high-risk patient group, i.e., those patients whose Tg failed to decrease to nondetectable levels and/or who exhibited a rise in Tg levels during the follow-up period.[9]

After excision of malignant thyroid tissue, virtually all patients are maintained on suppressive doses of thyroid hormones to prevent TSH-dependent recurrence of the cancer. The degree of suppression may vary, but in cases of metastatic differentiated thyroid cancers, consideration may be given to suppress patients to the limit of clinical tolerance.[4] However, deleterious side effects are associated with the chronic hyperthyroidism to which these patients are subjected. Studies have demonstrated that Tg levels respond to adjustments in T4 suppressive therapy. Optimum treatment regimes can therefore be identified as those which minimize circulating Tg levels.[9] Such an approach avoids excessive suppression, beyond the degree required to minimize Tg levels.

Determination of Tg: A Caveat

The major problem with Tg determinations is the interference caused by the presence of autoantibodies to Tg (anti-Tg Abs), reported to be present in approximately 26 percent of thyroid cancer patients.[9] The extent and type of interference caused by anti-Tg Abs is method specific.[8] Overestimation of Tg is typical of most competitive immunoassays and underestimation is characteristic of noncompetitive, immunometric “sandwich”-type assays. Spiking recovery procedures have been used to identify specimens in which the interference is significant. However, this approach has been criticized for being unreliable, with the demonstration that recovery studies did not identify all samples which yielded falsely low or nondetectable Tg results in thyroid cancer serum specimens.[7,8,9] Spencer[8] has recommended that until a reliable test is available for identifying significant interference caused by anti-Tg Abs in Tg determinations, Tg results not be reported for any sera containing anti-Tg Abs.

Antithyroglobulin Autoantibodies (Anti-Tg Abs)

Pathophysiology

Patients with a genetic predisposition to thyroid autoimmune diseases commonly develop antithyroid autoantibodies against thyroid antigens such as thyroid peroxidase (TPO, the microsomal antigen), TSH receptors and thyroglobulin. Anti-TSH receptor antibodies are associated with the frank hyperthyroidism of Graves’ disease (autoimmune thyrotoxicosis), and anti-TPO Abs (previously referred to as antimicrosomal antibodies) are associated with Hashimoto's disease, in which the autoimmune process gradually destroys thyroid tissue, eventually resulting in primary hypothyroidism. Although anti-Tg Abs do tend to appear in all thyroid autoimmune diseases, their presence is not disease specific.

Clinical Utility Of Anti-Tg Ab Determinations

Historically, anti-Tg Ab assays were used in conjunction with anti-TPO Ab assays to maximize the probability of a positive result in patients with autoimmune thyroid disease.[1,10] The original hemagglutination assays have now been largely replaced with far more sensitive and specific immunoassays. Results obtained with anti-TPO immunoassays were positive in virtually all cases of Hashimoto's disease and in approximately 85 percent of Graves' disease cases.[2,10] In contrast, the anti-Tg Ab tests were less frequently positive. Anti-Tg Ab tests now rarely add to the diagnostic information already obtained with an appropriately sensitive anti-TPO Ab assay, and the trend is to abandon the routine performance of these assays in tandem.

Anti-Tg immunoassays are now used primarily to screen specimens which are to be assayed for Tg.[2] However, the presence of anti-Tg Abs may have prognostic significance in thyroid-ablated thyroid cancer patients.[7] Antigenic stimulation should cease once the thyroid has been completely ablated, and autoantibody production is therefore expected to decrease with time. Retrospective studies have demonstrated that anti-Tg Ab concentrations do decline and disappear over time in patients who are judged to be disease free after thyroid ablation for differentiated thyroid cancer.[6] In contrast, anti-Tg Ab concentrations remain unchanged or exhibit an increase among patients with persistent or progressive thyroid cancer. These findings present the possibility of an intriguing new application for anti-Tg Abs as markers for differentiated thyroid cancer.

DPC Assays

DPC has released assays for thyroglobulin in the IMMULITE®* and Double Antibody† formats, assays for antithyroglobulin antibodies in the IMMULITE‡ and Milenia®† formats, an IMMULITE Thyroglobulin control* and an IMMULITE Thyroid Autoantibody control. IMMULITE§ and Milenia† assays for anti-TPO antibodies are also available. For further information, please fax (213) 776-0204 or contact your local DPC Representative.

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* Available outside the US.
† Available outside the US for clinical use. In the US, for research use only; not for diagnostic procedures.
‡ Under development.
§ Available worldwide.

DPC®

Diagnostic Products Corporation
5700 West 96th Street
Los Angeles, CA 90045-5597
Tel: 1 (800) 372-1782
Tel: 1 (213) 776-0180
Fax: 1 (213) 776-0204
Internet: http//www.dpcweb.com