Thyroglobulin

Thyroid Cancer – Diagnosis, Therapy & Follow Up
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Thyroid Cancer – Diagnosis, Therapy & Follow Up

Thyroglobulin monitoring after treatment of well-differentiated thyroid cancer.

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AIMS: The prognosis for well-differentiated thyroid carcinomas is favorable after treatment, but the rate of recurrence is around 20%. Cervical ultrasonography, radio-iodine scans, and monitoring of serum thyroglobulin (Tg) levels allow these recurrences to be diagnosed. The management of patients with isolated elevated Tg levels is controversial in the presence of negative radio-iodine scans.

METHODS: The records of 57 patients diagnosed with recurrence of well-differentiated thyroid cancer were reviewed. Serum Tg was not evaluated in 31 of these patients (group 1) and measured in the other 26 cases (group 2). RESULTS: Forty-three recurrence sites were found; four deposits in the thyroid bed and 39 cervical metastatic nodes, with an average of five nodes per patient. The radio-iodine scan was accurate in detecting 10/24 of cases, radiology in 9/17, and elevated Tg levels in 20/25. Thirteen patients with recurrences diagnosed on the basis of Tg levels had negative radio-iodine scans. After surgery, Tg levels were normal in 10 patients from group 1 and 16 patients from group 2 (p=0.0078). CONCLUSIONS: Elevated Tg levels are indicative of disease progression or recurrence in patients who have previously been operated on for well-differentiated thyroid cancer. Even when the radiological study or radio-iodine scan is normal, surgical re-exploration of the neck, with total thyroidectomy and lymphadenectomy, is advisable.


Diagnostic value of serum thyroglobulin measurements in the follow-up of differentiated thyroid carcinoma, a structured meta-analysis.

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OBJECTIVE: To investigate to what extent thyroid remnant ablation and withdrawal from thyroxine are required to achieve sufficient accuracy of serum thyroglobulin (Tg) measurements as an indicator of tumour recurrence in the follow-up of patients with differentiated thyroid carcinoma. DESIGN AND METHODS: We conducted a meta-analysis of the literature from 1975 to 2003 on serum Tg measurements in the follow-up of differentiated thyroid carcinoma. In a computer-based search, we initially found 915 articles that were finally narrowed down to 120. These 120 papers were subjected to strict in/exclusion criteria, leaving 46 articles (totaling 9094 patients). Data from these articles were extracted in a structured fashion and were grouped according to initial therapy, TSH status, Tg assay method and definition of a 'gold standard'. Original 2 x 2 tables were pooled by summary receiver operating characteristic curve analysis (sROCs), best estimates of sensitivity and specificity being obtained by the combination of sROCs and Mantel-Haenszel odds ratios. RESULTS: Despite considerable differences between series in laboratory and clinical methodology, we consistently found higher specificity for Tg measurements after thyroid remnant ablation than after surgery alone. Highest pooled sensitivity 0.961 +/- 0.013 (SE) was found for immunometric assay (IMA) after thyroid remnant ablation and thyroid hormone withdrawal, at a specificity of 0.947 +/- 0.007. Pooled sensitivity decreased significantly if ablated patients were tested while on thyroid hormone (0.778 +/- 0.023, at a specificity of 0.977 +/- 0.005). Significantly decreased pooled specificity was found in patients who did not undergo remnant ablation (sensitivity 0.972 +/- 0.023, at a specificity of 0.759 +/- 0.028). If recombinant human TSH (rhTSH) stimulation was used as a substitute for thyroxine withdrawal, sensitivity remained high (0.925 +/- 0.018) while specificity decreased to 0.880 +/- 0.013. In all analyses, specificity of Tg would decrease when unspecified activity in the thyroid region at scintigraphy was considered benign, whereas sensitivity decreased when such activity was considered malignant. CONCLUSION: This study confirms that the best accuracy of Tg-guided follow-up in patients treated for differentiated thyroid carcinoma is obtained if treatment includes remnant ablation, and Tg testing is performed while off thyroxine.


Treatment of iodine-negative thyroglobulin-positive thyroid cancer: differences in outcome in patients with macrometastases and patients with micrometastases.

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PURPOSE. During the follow-up of patients with well-differentiated thyroid carcinoma, some patients have elevated serum thyroglobulin (Tg) levels without any evidence of radioiodine accumulation on diagnostic whole-body scan (d-WBS). The treatment strategy in these patients is considered a clinical dilemma, with some groups recommending blind use of high-dose radioiodine therapy. The aim of this study was to evaluate whether or not high doses of radioiodine have beneficial effects in these patients. METHODS. Twenty-seven patients were included in the study. All patients had negative d-WBS and elevated levels of Tg. All received high doses of radioiodine. The mean follow-up period was 6.3+/-.5.8 years. There were 11 patients with macrometastases and 16 with micrometastases. RESULTS. Post-treatment WBS revealed radioiodine accumulation in 19 of 24 (79%) patients. Serum Tg levels were decreased in 8 of 16 (50%) patients. Among patients with micrometastases, five out of seven (71%) demonstrated a decrease in serum Tg levels. Among patients with macrometastases, three out of nine (33%) demonstrated a decrease in Tg values and three (33%) have died due to metastatic thyroid cancer. CONCLUSION. Radioiodine treatment may have a beneficial therapeutic effect in patients who have elevated levels of serum Tg and negative d-WBS. This is especially true in those patients with micrometastases; in patients with macrometastases, a beneficial effect of this approach may be observed less frequently.
The early detection of recurrent differentiated thyroid carcinoma (DTC) cells in the post-surgery DTC patients relies on the sensitivity of measuring both the level of thyroglobulin (Tg) and 131-Iodine distribution by Whole Body Scan (WBS). Undetectable level of Tg associated with negative WBS or elevated levels of Tg associated with positive WBS ("discordant") is ordinarily indicative of either absence or presence of disease. At times, elevated level of Tg with negative WBS or low levels of Tg with positive WBS ("discordant") could also occur. In the present study, we retrospectively reviewed series of 573 patients with DTC followed in the Diagnostic Imaging and Radiotherapy of the University "Federico II" of Naples between 1993 and 1997. We focused on 9 out of 573 patients (1.56%) who had a discordant pattern with low level of Tg/positive WBS in the post-surgical follow-up. Four patients were metastatic at presentation while 5 patients with metastases during follow-up still remained in persistently low levels of Tg (<5 ng/mL). This result does point to some flaw in the evaluation of "discordant" cases. Reviewing data previously described series by resetting cut-off values of Tg <1 ng/ml as undetectable changed the apparent "discordant" subgroup of patients into "concordant". Recent introduction of recombinant human TSH (rhTSH) to enhance the expression level of Tg brought significant increase in the sensitivity of diagnostic evaluation of thyroid cancer patients. The role of burdensome WBS in the follow up evaluation of DTC patients is significantly reduced over time especially in low-risk patients while the relevance of Tg assay is steadily increased. Sensitive Tg assays, significantly improved our ability to assess disease status in follow-up of DTC. Given the possibility of late disease relapses, the need for long-term follow-up, and reduced delay in treatment of persistent disease, there is still need for greater sensitive diagnostic tools for DTC.

Predictive value for disease progression of serum thyroglobulin levels measured in the postoperative period and after (131)I ablation therapy in patients with differentiated thyroid cancer.


The aim of our study was to evaluate and compare in thyroid cancer patients the predictive value for disease progression of serum thyroglobulin (Tg) levels measured under thyroid-stimulating hormone (TSH) stimulation, in the postoperative period just before (131)I ablation therapy and at the time of control 6-12 mo later. METHODS: Two-hundred-twelve consecutive patients treated for a well-differentiated thyroid carcinoma (184 papillary, 28 follicular) with no initial distant metastases were retrospectively studied. All patients had a total or near-total thyroidectomy followed by ablation with 3.7 GBq (131)I. Tg levels were determined just before ablation therapy (Tg1) and 6-12 mo later (Tg2). Thresholds of 30 and 10 ng/mL were used for Tg1 and Tg2, respectively. Univariate and multivariate analyses were performed to assess the predictive value for disease progression of the 2 Tg determinations. RESULTS: Thirty patients had a Tg1 level > 30 ng/mL. Six to 12 mo later, 30 patients had a Tg2 level > 10 ng/mL, 19 of whom had initially a Tg1 level > 30 ng/mL. Disease progression was reported in 20 patients (9%). Progression-free survival rates were significantly lower in patients with a low Tg1 or Tg2 level but the difference was more important with Tg2. With univariate analysis, 5 variables were significantly associated with disease progression: Tg2, Tg1, node invasion, extrathyroidal extension, and tumor size. With multivariate analysis, only Tg2 (odds ratio [OR] = 16.4; 95% confidence interval [95% CI] = 5.7-47.4; P < 0.001) and node invasion (OR = 2.7; 95% CI = 1.0-7.2; P = 0.04) had an independent predictive value. When only initial parameters were considered, Tg1 and node invasion were the 2 independent prognostic factors. The OR decreased for Tg1 (OR = 10.1; 95% CI = 4.0-25.7; P < 0.001) but increased for node invasion (OR = 4.4; 95% CI = 1.7-11.2; P = 0.002). CONCLUSION: Among all clinical and tumoral variables, lymph node invasion and serum Tg levels are 2 important parameters to define the risk of disease progression. Although Tg2 appears more significant than Tg1, both Tg levels measured under TSH stimulation, in the postoperative period and a few months after ablative therapy, have a predictive value. In clinical practice, patients at risk can be selected as soon as the initial lymph node status and Tg1 level are known.


Prognostic value of thyroglobulin serum levels and 131I whole-body scan after initial treatment of low-risk differentiated thyroid cancer.

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Diagnostic iodine-131 whole-body scan ((131)I-WBS) and serum thyroglobulin values (Tg) performed 6 to 12 months after thyroid ablation for differentiated thyroid carcinoma were evaluated in 194 consecutive patients at the Hospital de Navarra, (Pamplona, Spain). All patients underwent near-total thyroidectomy and (131)I ablation with 3.7 GBq. Patients with positive anti-Tg antibodies or with (131)I uptake outside the neck were previously excluded. Uptake of (131)I in the thyroid bed was detected in 27 patients (13.9%). Serum Tg levels were below 0.5 ng/mL in 133 patients, ranged from 0.5-10 ng/mL in 39 patients, and was above 10 ng/mL in 22 patients. After a follow-up of 7.7 +/- 3.3 years, persistence of the illness has been observed in 2 patients with undetectable Tg (1.5%), but metastases were not detected in any case. In those with Tg higher than 0.5 ng/mL, 29 of 61 patients had persistence of the disease (47.5%) with evidence of metastases in 15 (24.5%), irrespective of the initial total body scan (131)I uptake. In conclusion, serum Tg levels obtained after thyroid ablation has a good prognostic value and permits the selection of patients for further diagnostic studies, while diagnostic (131)I-WBS performed at that time did not correlate with results of Tg and scarcely provides additional information.
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Thyroglobulin: a specific serum marker for the management of thyroid carcinoma.

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Thyroglobulin measurements in tissue and serum play an integral role in the evaluation of patients who have thyroid cancer. Immunohistochemical detection of thyroglobulin in surgical specimens is useful in the differential diagnosis of tumors of unknown origin; however, the most important application of thyroglobulin measurement in clinical practice is in the postsurgical management of differentiated thyroid carcinoma. Serum thyroglobulin is a highly specific and sensitive tumor marker for detecting persistent or recurrent thyroid carcinoma and for monitoring clinical status. The reappearance of circulating thyroglobulin after total thyroid ablation is pathognomonic for the presence of tumor. The measurement of thyroglobulin in serum is challenging, however, and several analytical problems limit assay performance. Thyroglobulin autoantibody interference is a particularly significant concern that requires all thyroglobulin samples to be screened for their presence. No immunoassay is totally free from interference by thyroglobulin autoantibodies. Measurement of thyroglobulin mRNA to detect circulating tumor cells may help to overcome some of the limitations of current protein-detection methods; serum thyroglobulin will continue to remain the “golden standard.” The complex functional features of thyroid carcinomas make sole reliance upon any one diagnostic technique, including thyroglobulin assessments, potentially misleading. Thyroglobulin measurements are a critical component of a multifaceted diagnostic approach to this disease.


The influence of thyroglobulin on functional imaging in differentiated thyroid cancer.

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AIM: Recombinant human thyroid stimulating hormone (rhTSH) for an exogenous stimulation of TSH and consequent thyroglobulin (hTG) synthesis has reinitiated a discussion about the usefulness of diagnostic procedures for the follow-up of differentiated thyroid cancer (DTC). METHOD: Fifty consecutive patients with DTC who received whole-body iodine scintigraphy (WBS) and positron emission tomography (PET) were evaluated. RESULTS: The work-up was normal in 18/50. In 32 patients, functional imaging detected DTC. In 44% exogenous TSH stimulation with rhTSH was used and thyroxin was withdrawn in the others. The hTG under stimulation ranged from 0.8 to 5.004 ng x ml(-1). It was below 2 ng x ml(-1) in four (12.5%) patients. In total, 91 tumour sites were identified by positron emission tomography (PET) and 47 sites by WBS. PET and WBS showed corresponding uptake in 38% of lymph node, 48% of parenchymal and 43% of bone metastases. PET detected additional 53% of lymph node (WBS 9%), 38% of parenchymal (WBS 14%) and 28.5% of bone metastases. CONCLUSION: It is concluded that PET is more sensitive than WBS for the detection of DTC. The follow-up of DTC patients with hTG levels alone misses a significant number of true positive cases. Its use should therefore be restricted to selected low risk patients only.

Thyroid. 2003 Dec;13(12):1163-7.

Diagnostic utility of thyroglobulin detection in fine-needle aspiration of cervical cystic metastatic lymph nodes from papillary thyroid cancer with negative cytology.


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Cystic changes in metastatic cervical lymph nodes (CLN) from papillary thyroid cancer (PTC) may be a diagnostic pitfall in fine-needle aspiration biopsy (FNAB) cytology. We investigated in a series of CLN metastases from thyroid cancers (TC), including cystic PTC, and from a wide spectrum of extrathyroidal malignancies, the diagnostic role for metastatic TC of the rapid detection of thyroglobulin in eluates from FNAB (FNAB-Tg) of CLN. The study was carried out in a group of 79 subjects (22/57 M/F; median age, 56 years; range, 20-86 years) with enlarged CLN and thyroid nodules (TN), examined for potential metastatic TC, and harboring a large spectrum of incidentally diagnosed extrathyroidal malignancies (n = 24, mostly represented by lymphomas, lung, and breast cancers), CLN metastases from thyroid cancers (n = 28, including 6 cystic metastatic PTC), 6 specific lymphadenitis and 21 reactive lymphadenitis mostly detected (n = 16) during follow-up of patients with previously ablated TC. Markedly high FNAB thyroglobulin (Tg) values were found in all metastatic CLN TC. Two of the six cases with cystic metastatic CLN PTC were diagnosed by FNAB-Tg but not by cytology. In conclusion, FNAB-Tg has been confirmed as an easy modality and fast procedure to diagnose CLN metastasis from TC and high FNAB-Tg values with nondiagnostic cystic cytology strongly suggest cystic metastatic PTC.


Serum thyroglobulin as a marker of thyroid neoplasms after childhood cancer.


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AIM: To evaluate serum thyroglobulin (Tg) level as a marker of the development of thyroid disease when following individuals who received neck irradiation therapy in childhood. METHODS: In a non-randomized cross-sectional study Tg was assessed in 172 survivors of childhood cancer 10.8 y (1.9-24) median (range) after diagnosis and 7.9 y (0.9-24.3) median (range) after the end of treatment. The patients were divided into two groups: group 1 included 47 patients who had received irradiation to the neck and group 2 included 125 patients who did not receive irradiation to the neck. RESULTS: Patients who had received irradiation to the neck had...
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significantly higher Tg levels compared with those who did not receive neck irradiation: median 14.0 (1.0-189.0) microg/L vs median 8.8, (0.7-112.2) microg/L (p < 0.001). Six out of seven patients with elevated Tg levels (>70 microg/L) had received neck irradiation. Among these six patients, two patients developed secondary differentiated thyroid cancer and two patients developed benign thyroid neoplasms. None of the patients who had normal levels of Tg developed thyroid cancer. CONCLUSION: A high Tg level should be a cause for further investigation in the follow-up of individuals who have received irradiation therapy in childhood.


Technetium-99m tetrofosmin single photon emission computed tomography to detect metastatic papillary thyroid carcinoma in patients with elevated human serum thyroglobulin levels but negative I-131 whole body scan.

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AIM: The aim of this study was to evaluate the effectiveness of technetium-99m tetrofosmin (Tc-99m TF) single photon emission computed tomography (SPECT) of the neck and chest to detect metastatic lesions in papillary thyroid carcinoma (PTC) after near total thyroidecmy and radioiodine (I-131) treatment in patients who present with elevated serum human thyroglobulin (hTg) levels but negative I-131 whole body scan (WBS).

MATERIALS AND METHODS: Twenty patients with PTC treated by near total thyroidecmy and I-131 treatments were included in this study. All 20 patients had negative I-131 WBS results and elevated hTg levels (hTg 2.0 microIU/ml) under thyroid-stimulating hormone (TSH) stimulation (TSH 30 microIU/ml). Nineteen of the 20 cases were confirmed to have metastases by operation/biopsy histopathological findings or clinical follow-up longer than 1 year by additional morphological imaging techniques. The remaining patient has been followed up closely and has been disease free for 10 months. Tc-99m TF SPECT was performed to detect metastatic lesions.

RESULTS: Twenty patients with PTC treated by near total thyroidecmy and I-131 treatments were included in this study. All 20 patients had negative I-131 WBS results and elevated hTg levels (hTg 2.0 microIU/ml) under thyroid-stimulating hormone (TSH) stimulation (TSH 30 microIU/ml). Nineteen of the 20 cases were confirmed to have metastases by operation/biopsy histopathological findings or clinical follow-up longer than 1 year by additional morphological imaging techniques. The remaining patient has been followed up closely and has been disease free for 10 months. Tc-99m TF SPECT was performed to detect metastatic lesions.

CONCLUSIONS: While Tc-99m TF SPECT is a useful additional tool to detect metastatic lesions in PTC with elevated hTg but negative I-131 WBS, however, smaller lymph nodes and miliary lung metastases may be missed.


Three-week thyroxine withdrawal thyroglobulin stimulation screening test to detect low-risk residual/recurrent well-differentiated thyroid carcinoma.

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Measurement of serum TSH-stimulated thyroglobulin (Tg) is recognized as a sensitive method for detecting residual/recurrent well-differentiated thyroid carcinoma (WDTC) in patients previously treated by surgery and radioactive iodine (RAI) ablation therapy. WDTC patients who have an undetectable serum Tg on thyroid hormone therapy (THT) in the absence of Tg-antibody interference are considered to be at low risk for residual/recurrent disease. Traditional management has been to withdraw T4 for 4-6 weeks or T3 for 3 days to stimulate endogenous TSH. However, this prolonged THT withdrawal induces hypothyroidism and its concomitant morbidity. In the present study, we assess the efficacy of shortening the time of T4 withdrawal to only 3 weeks for detecting residual/recurrent WDTC as a sufficient serum TSH stimulus for obtaining a positive serum Tg result without a routine diagnostic whole body scan (WBS).

Additionally, we have evaluated the impact of such a T4 withdrawal interval on quality of life and loss of employment time. A total of 181 patients with WDTC selected for study had previously been treated with a bilateral surgical thyroidectomy followed by RAI ablation therapy (average post-surgery to follow-up interval of 10.8 yr). All of the cohort had an undetectable (<1 microg/l) serum Tg on THT without Tg-antibody interference. Serum TSH and Tg were measured before and after cessation of T4 therapy for 3 weeks. A serum Tg > or = 2 microg/l was considered positive for residual/recurrent disease. A quality of life questionnaire [Short-Form 36 (SF-36)] was administered before withdrawal, at peak TSH and after resumption of therapy. From the completed SF-36 questionnaires, the overall degree of functional impairment was not severe and did not result in loss of employment time. Moreover, this protocol identified three possible responses to the 3-week T4 withdrawal interval as follows: a) serum Tg undetectable with TSH > or = 25 mIU/l (approximately 75% of total cohort); b) serum Tg > or = 2 microg/l (approximately 10% of total cohort) which will require further investigation and treatment for residual/recurrent disease; c) undetectable serum Tg with inadequate TSH rise (approximately 15% of total cohort), which will require TSH stimulation by either longer T4 withdrawal or recombinant human TSH to exclude residual disease.

We conclude that a stimulated serum Tg test performed 3 weeks after T4 withdrawal is a simple and cost-effective first-line screening test with minimal morbidity which is sufficient to evaluate low-risk WDTC patients for recurrent/residual carcinoma.


Thyroglobulin: current aspects of its role in autoimmune thyroid disease and thyroid cancer.

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Thyroglobulin (Tg) is a large glycoprotein (molecular weight: 660000) with 2 polypeptide chains of approximately 2768 amino acids each. It functions both as a pro-hormone and storage hormone for thyroid hormones. The complete Tg gene sequence has been determined for human, rat and bovine species. Tg is one of the thyroid autoantigens recognised in patients with autoimmune thyroid disease (AITD). Antibodies to Tg (TgAb) are present in the serum of patients with AITD and are also sometimes present in healthy euthyroid subjects. Though at least 40 antigenic epitopes on human Tg have been identified, only 2 or 3 of these bind TgAb. Epitope mapping studies suggest that TgAb in AITD patients express a restricted binding pattern while TgAb in the serum of healthy individuals do not show such specific binding. There is evidence to suggest that iodination of Tg may alter these epitope binding patterns. TgAb IgG on the other hand, do not appear to be subclass restricted. Several Tg fragments capable of inducing a T-cell response have been described. Tg is routinely used in the postoperative monitoring of patients with differentiated thyroid cancer. Its use has been limited by
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provides with assay methods which include poor inter-laboratory standardisation, poor inter-assay variation, low functional sensitivity of the assays, hook effects, and interference from TgAb present in patients serum. The use of rhTSH in stimulating Tg prior to testing has improved the sensitivity of Tg values in the suppressed state. Autoimmunity. 2003 Sep-Nov;36(6-7):423-8.

A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma.

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Recombinant human thyrotropin-stimulated serum thyroglobulin combined with neck ultrasonography has the highest sensitivity in monitoring differentiated thyroid carcinoma.


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Recombinant human TSH (rhTSH) stimulated Tg measurement and (131)I whole body scan (WBS) have been validated as informative tests in the postsurgical follow-up of differentiated thyroid carcinoma. We report the diagnostic accuracy of Tg measurement and diagnostic WBS, alone or in combination, after rhTSH stimulation in a retrospective, consecutive series of patients undergoing follow-up for differentiated thyroid cancer. Routine procedures also include neck ultrasound in every patient and post-therapy WBS when indicated. We studied 340 consecutive patients with differentiated thyroid carcinoma, previously treated with near-total thyroidectomy and (131)I thyroid ablation, scheduled for routine diagnostic tests. At baseline on L-T(4)-suppressive therapy, 294 patients had undetectable (<1 ng/ml) serum Tg and negative anti-Tg autoantibodies (TgAb), 25 patients had undetectable serum Tg and positive TgAb, and 21 patients had detectable serum Tg and negative TgAb. These patients were tested for the presence of active disease by rhTSH stimulation. The results of our study showed that rhTSH-stimulated Tg alone had a diagnostic sensitivity of 85% for detecting active disease and a negative predictive value (NPV) of 98.2%. After adding the results of neck ultrasound, sensitivity increased to 96.3%, and the NPV to 99.5%. rhTSH-stimulated WBS had a sensitivity of only 21% and a NPV of 89%. The combination of rhTSH-stimulated Tg and WBS had a sensitivity of 92.7% and a NPV of 99%. We conclude that the rhTSH-stimulated Tg test combined with neck ultrasonography has the highest diagnostic accuracy in detecting persistent disease in the follow-up of differentiated thyroid carcinoma. A detectable level of serum Tg on L-T(4), its conversion from undetectable to detectable after rhTSH, and/or a suspicious finding at ultrasound will allow the identification of patients requiring therapeutic procedures without the need for diagnostic WBS.

Thyroid. 2003 Aug;13(8):833-4; author reply 834.

Thyroglobulin-positive, radiiodine-negative thyroid cancer.

Gemsenjager E.

Treatment of iodine negative, thyroglobulin positive, thyroid cancer patients: do we miss the target when we shoot in the dark?

Kabasakal L.
Antithyroglobulin antibodies can interfere with the measurement of thyroglobulin yielding spuriously high or low levels depending on the method used. Interference is unrelated to the antibody concentration and can occur at very low concentrations. We report a patient in whom antithyroglobulin antibodies below the cut-off for positivity nearly led to an incorrect diagnosis of thyrotoxicosis factitia.


Outcome in patients with differentiated thyroid cancer with negative diagnostic whole-body scanning and detectable stimulated thyroglobulin.

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BACKGROUND: Management of patients with differentiated thyroid carcinoma with negative diagnostic radiiodine scanning and increased serum thyroglobulin (Tg) concentrations is a widely debated problem. High-dose iodine-131 treatment of patients who have a negative (131)I diagnostic whole-body scan (WBS) is advocated. However, the therapeutic benefit of this “blind” treatment is not clear. OBJECTIVE: To investigate the course of serum Tg during thyroid hormone suppression therapy (Tg-on) and clinical outcome in patients with negative diagnostic (131)I scanning and increased serum Tg concentrations during thyroid hormone withdrawal (Tg-off), after treatment with high-dose (131)I. DESIGN: Retrospective single-center study. METHODS: Fifty-six patients were treated with a blind therapeutic dose of 150 mCi (131)I. Median follow-up from this treatment until the end of observation was 4.2 Years (range 0.5-13.5 Years). RESULTS: The post-treatment WBS revealed (131)I uptake in 26 patients, but none in the remaining 28 patients. In this study the Tg-on values did not change after treatment in either the positive or the negative post-treatment WBS group. During follow-up, 18 of the 28 patients with a positive post-treatment WBS achieved complete remission, compared with 10 of the 28 patients with a negative post-treatment WBS. Nine patients in the negative group died, but no patients died in the positive post-treatment group (P=0.001). CONCLUSIONS: High-dose iodine treatment in diagnostically negative patients who have a negative post-treatment scan seems to confer no additional value for tumor reduction and survival. In patients with a positive post-treatment scan, high-dose iodine treatment can be used as a diagnostic tool to identify tumor location, and a therapeutic effect may be present in individual cases.
The diagnostic use of the rhTSH/thyroglobulin test in differentiated thyroid cancer patients with persistent disease and low thyroglobulin levels.


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BACKGROUND: Serum thyroglobulin (Tg) measurement after TSH stimulation, by either thyroid hormone withdrawal or recombinant human TSH (rhTSH) administration, is the most sensitive method for early detection of patients with persistent or recurrent differentiated thyroid cancer (DTC) after total thyroidectomy and 131I ablation. The use of rhTSH is now increasing because it avoids thyroid hormone suppressive therapy (THST) withdrawal and the consequent symptoms of severe hypothyroidism. Current guidelines suggest measurement of serum Tg 4 days after starting a 2-day course of rhTSH injections, and assumes that Tg reaches maximum serum levels at that time. OBJECTIVE: The present study was carried out to evaluate the accuracy of rhTSH/thyroglobulin test in DTC patients with persistent disease and low thyroglobulin levels. PATIENTS AND MEASUREMENTS: A series of 13 DTC patients was selected because they had proven persistent disease associated with low Tg levels (<2.0 micro g/l) under 1-thyroxine treatment. In all of them, serum Tg was > 5.0 micro g/l at the last THST withdrawal. We measured serum Tg and TSH levels on days 0.5, 1, 1.5, 2, 4, 7, 10 and 15 after the first of a 2-day course of intramuscular rhTSH injections. RESULTS: Serum Tg values were variable in terms of both peak and time-course. Detectable serum Tg levels were recorded on day 4 in all patients. However, among these 13 patients, the peak Tg value was reached earlier than day 4 in three patients and later in two others. In one patient, Tg level at day 2 was higher (3.0 micro g/l) than at day 4 (1.8 micro g/l). In six of the 13 patients we compared Tg values after rhTSH to those subsequently obtained after THST withdrawal: in five of them Tg values were two to three times higher after the latter stimulation. Serum Tg value variability after rhTSH was partially accounted for by variability of serum TSH levels, which were inversely related to patient body surface. CONCLUSIONS: In DTC patients with persistent disease and low Tg levels, optimization of the diagnostic use of Tg measurement after rhTSH may require rhTSH dose adjustment to the patient body surface area and repeated blood sampling, in order to improve diagnostic accuracy. In these patients not even a TSH-stimulated serum Tg cut-off of 2.0 micro g/l on day 4 provides 100% accuracy, whereas a cut-off of 1.0 micro g/l seems more appropriate. Therefore, in this subset of patients, if any detectable Tg level > or = 1.0 micro g/l is found after rhTSH re-evaluation after THST should be advised.


F18-fluorodeoxyglucose positron emission tomography in detecting metastatic papillary thyroid carcinoma with elevated human serum thyroglobulin levels but negative I-131 whole body scan.

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Papillary carcinomas are the most common thyroid malignancies. They invade the regional lymphatics and metastasize frequently to local lymph nodes in the neck. Distant metastasis, generally to the lungs, is also common. METHODS: The aim of this study is to evaluate the effectiveness of F18-fluorodeoxyglucose (FDG) positron emission tomography (PET) to detect metastatic lesions in patients with papillary thyroid carcinomas (PTC) after nearly total thyroidectomy and I-131 treatments who present with elevated human serum thyroglobulin (hTg) levels but negative I-131 whole body scan (WBS). Twenty patients with PTC who underwent nearly total thyroidectomy and radioiodine treatments were included in this study. RESULTS: All of the 20 patients had negative I-131 WBS results and elevated hTg levels (hTg > or = 2.0 microIU/ml) under thyroid-stimulating hormone (TSH) stimulation (TSH > or = 30 microIU/ml). CONCLUSIONS: FDG-PET was performed to detect metastatic lesions. F18-fluorodeoxyglucose-PET could detect hypermetabolic lesions in 17 patients but failed to demonstrate malignant pulmonary metastases in two patients. No definite lesion was found in FDG-PET. X-ray chest computed tomography (CT) and other imaging studies of the remaining one patient. This study showed that FDG-PET is a useful tool in detecting metastatic lesions in PTC with elevated hTg but negative I-131 WBS. However, miliary lung metastases may be missed in FDG-PET. In this circumstance, chest CT should be included in the follow-up protocol.


Effects of therapeutic doses of 131I in thyroid papillary carcinoma patients with elevated thyroglobulin level and negative 131I whole-body scan: comparative study.

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OBJECTIVE: Previous studies have shown a high rate of visualization of uptake and a decrease in serum thyroglobulin (Tg) after therapeutic doses of 131I in well-differentiated thyroid cancer patients with elevated thyroglobulinemia but negative diagnostic 131I whole-body scan (DxWBS), but its therapeutic effect remains controversial. We evaluate the effect of therapeutic doses of 131I in patients with elevated thyroglobulin level but negative DxWBS. DESIGN: Among papillary thyroid carcinoma patients who underwent total or near-total thyroidectomy and remnant ablation with radioiodine during 1996 to 2000 in our hospital, the patients who showed elevated serum Tg levels and no abnormal uptake in DxWBS were selected. The selection for treatment or no treatment was decided according to the preference of the patients, considering side-effects of therapeutic doses of 131I, and the patients were thereafter studied retrospectively. PATIENTS: Sixty papillary thyroid carcinoma patients with elevated thyroglobulinemia but negative DxWBS were included. Twenty-eight patients were treated, and 32 were untreated. MEASUREMENTS: We compared serum Tg levels measured at less than 3 months before the administration of therapeutic doses of 131I or DxWBS with the levels at 6-12 months after administration between two groups. Comparable data on changes in serum Tg levels during TSH suppression (Tg-on) and those in hypothyroid phase (Tg-off) were available in 25 and 49 patients, respectively. RESULTS: Percentage decreases in both Tg-on and Tg-off levels of the treated group [41.2 (10.1-94.1)% and 37.0 (-176.6-68.4)%], respectively] were significantly higher than those of the untreated group [-43.6 (-180.1-7.3)% and -66.6 (-1064.2-39.1)%, respectively] (P < 0.001). The treated patients were followed-up for 23.8 +/- 19.6 months.
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months after the administration of therapeutic doses of 131I. In four cases, serum Tg levels converted to negative (< 1.0 ng/ml) both on and off T4 15-22 months after the administration of therapeutic doses of 131I, and negative serum Tg levels persisted for 24-70 months. However, negative conversion of elevated serum Tg levels was not observed in any of the untreated group. Post-treatment WBS revealed pathologic uptake in 12 of 28 cases (42.9%). CONCLUSIONS: This study revealed that the administration of therapeutic doses of 131I has a therapeutic effect, at least for palliation in short-term observation, considering the serum Tg level as an index of tumour burden, and that it can disclose previously undiagnosed lesion in some patients with differentiated thyroid cancer who show elevated thyroglobulin level but negative diagnostic 131I whole-body scan.

J Clin Endocrinol Metab. 2003 Apr;88(4):1433-41.

A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma.

Mazzaferrini EL, Robbins RJ, Spencer CA, Braverman LE, Pacini F, Wartofsky L, Haugen BR, Sherman SI, Cooper DS, Braunstein GD, Lee S, Davies TF, Arafah BM, Ladenson PW, Pinchera A.

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Recent studies have provided new information regarding the optimal surveillance protocols for low-risk patients with differentiated thyroid cancer (DTC). This article summarizes the main issues brought out in a consensus conference of thyroid cancer specialists who analyzed and discussed this new data. There is growing recognition of the value of serum thyroglobulin (Tg) as part of routine surveillance. An undetectable serum Tg measured during thyroid hormone suppression of TSH (THST) is often misleading. Eight studies show that 21% of 784 patients who had no clinical evidence of tumor with baseline serum Tg levels usually below 1 micro g/liter during THST had, in response to recombinant human TSH (rhTSH), a rise in serum Tg to more than 2 micro g/liter. When this happened, 36% of the patients were found to have metastases (36% at distant sites) that were identified in 91% by an rhTSH-stimulated Tg above 2 micro g/liter. Diagnostic whole body scanning, after either rhTSH or thyroid hormone withdrawal, identified only 19% of the cases of metastases. Ten studies comprising 1599 patients demonstrate that a TSH-stimulated Tg test using a Tg cutoff of 2 micro g/liter (either after thyroid hormone withdrawal or 72 h after rhTSH) is sufficiently sensitive to be used as the principal test in the follow-up management of low-risk patients with DTC and that the routine use of diagnostic whole body scanning in follow-up should be discouraged. On the basis of the foregoing, we propose a surveillance guideline using TSH-stimulated Tg levels for patients who have undergone total or near-total thyroideotomy and (131)I ablation for DTC and have no clinical evidence of residual tumor with a serum Tg below 1 micro g/liter during THST.


Positive predictive value of serum thyroglobulin levels, measured during the first year of follow-up after thyroid hormone withdrawal, in thyroid cancer patients.

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The follow-up of patients with papillary and follicular thyroid carcinoma after thyroideotomy and radioiodine ablation is mainly based on serum thyroglobulin (Tg) level determination. The positive predictive value (PPV) of serum Tg level after thyroid hormone withdrawal, measured during the first 12 months of follow-up (initial off L-T(4) Tg), was studied in 256 consecutive differentiated thyroid cancer patients. All underwent a total thyroideotomy and 3.7 GBq (131)I ablation; 37 patients had an elevated initial off L-T(4) Tg level. This study focuses on these 37 patients, 9 of whom had a clinical recurrence. The present data confirm that in this selected cohort of patients, 74-185 MBq (131)I-total body scan (TBS) has no clinical interest in the initial work-up and during the subsequent follow-up because it was negative in all patients, except in one with recurrent disease. The PPV of initial serum off L-T(4) Tg level above 5 ng/ml and 10 ng/ml was 42% and 53%, respectively; this PPV was only 50% at the time of recurrence or subsequent control. This relatively low PPV is related to the low recurrence rate in this series of patients, despite a prolonged follow-up, and to the subsequent decrease of serum Tg level in 14 of 37 (38%) patients in the absence of any further treatment. In contrast, the PPV of the increasing slope of serum Tg levels obtained after thyroid hormone withdrawal (83%) was excellent. In conclusion, we confirm that (131)I-TBS has a limited interest for the follow-up of thyroid cancer patients. Follow-up should rely on serum Tg level and prognostic parameters; however, initial serum Tg may be produced by thyroid tissues of various significance, an increase at two consecutive determinations indicating disease progression and a decrease being related to late effects of therapy. The best PPV is brought by the slope of serum Tg levels.


**Te-99m MIBI SPECT in detecting metastatic papillary thyroid carcinoma in patients with elevated human serum thyroglobulin levels but negative I-131 whole-body scan.**

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Department of Nuclear Medicine and Internal Medicine, Taipei Medical University Hospital, Taipei, Taiwan.

Papillary carcinomas are the most common thyroid malignancies. They invade the regional lymphatics and metastasize frequently to local lymph nodes in the neck. Distant metastasis, generally to the lungs, is also common. The aim of this study is to evaluate the effectiveness of single photon emission computed tomography (SPECT) with technetium-99m methoxyisobutylisonitrile (Te-99m MIBI) in detecting metastatic lesions in patients with papillary thyroid carcinoma (PTC) after nearly total thyroideotomy and radioiodine (I-131) treatment who present with elevated serum human thyroglobulin (hTg) levels but negative I-131 whole-body scan (WBS). METHODS: Twenty patients of PTC who underwent nearly total thyroideotomy and I-131 treatments were included in this study. All of the 20 patients had negative I-131 WBS results and elevated hTg levels (hTg > or = 20 microU/mL) under thyroid-stimulating hormone (TSH) stimulation (TSH > or = 30 microU/mL). Technetium-99m MIBI SPECT was performed to detect metastatic lesions. RESULTS: Technetium-99m MIBI SPECT demonstrated lesions in 10 patients. Technetium-99m MIBI SPECT
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failed to demonstrate lesions in nine patients including small lymph node and lung metastases. CONCLUSIONS: This study showed that Tc-99m MIBI SPECT is a useful tool to detect metastatic lesions in PTC with elevated hTg but negative I-131 WBS. However, small lymph node and lung metastases may be missed in Tc-99m MIBI SPECT. In the latter circumstance, other imaging studies should be included in the follow-up protocol.


Serum thyroglobulin and 131I whole body scan after recombinant human TSH stimulation in the follow-up of low-risk patients with differentiated thyroid cancer.


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OBJECTIVE: The standard postoperative follow-up of patients with differentiated thyroid cancer (DTC) has been based upon serum thyroglobulin (Tg) measurement and (131)I whole body scan ((131)I-WBS) after thyroid hormone (T4(4)) treatment withdrawal. However, (131)I-WBS sensitivity has been reported to be low. Thyroid hormone withdrawal, often associated with hypothyroidism-related side effects, may now be replaced by recombinant human thyrotropin stimulating hormone (rhTSH). The aim of our study was to evaluate the diagnostic accuracy of (131)I-WBS and serum Tg measurement obtained after rhTSH stimulation and of neck ultrasonography in the first follow-up of DTC patients. DESIGN: Ninety-nine consecutive patients previously treated with total thyroideectomy and (131)I ablation, with no uptake outside the thyroid bed on the post-ablative (131)I-WBS (low-risk patients) were enrolled. METHODS: Measurement of serum Tg and (131)I-WBS after rhTSH stimulation, and ultrasound examination (US) of the neck. RESULTS: rhTSH-stimulated Tg was <or=1 ng/ml in 78 patients (Tg-) and >1 ng/ml (Tg+) in 21 patients, including 6 patients with Tg levels >5 ng/ml. (131)I-WBS was negative for persistent or recurrent disease in all patients (i.e., sensitivity =0%). US identified lymph-node metastases (confirmed at surgery) in 4/6 (67%) patients with stimulated Tg levels >5 ng/ml, in 2/15 (13%) with Tg >1<5 ng/ml, and in 2/78 (3%) who were Tg-negative. CONCLUSIONS: (i) diagnostic (131)I-WBS performed after rhTSH stimulation is useless in the first follow-up of DTC patients; (ii) US may identify lymph node metastases even in patients with low or undetectable serum Tg levels.

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Predictive value of serum thyroglobulin after surgery for thyroid carcinoma.

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OBJECTIVE: To determine the relationship between stimulated serum thyroglobulin levels (taken 3 months after total thyroideectomy) and tumor stage and recurrence in patients with well-differentiated thyroid carcinoma. STUDY DESIGN: Retrospective chart review in a tertiary care institution. METHODS: Two hundred thirteen consecutive patients with well-differentiated thyroid carcinoma treated between 1983 and 1998 were identified. Data were collected on clinicopathological variables, stimulated serum thyroglobulin levels obtained 3 months after total thyroideectomy prior to 131I therapy and recurrence. RESULTS: A high postoperative thyroglobulin level was significantly associated with advanced-stage disease at presentation (P =.005, Kruskall-Wallis) but not with any of the other clinicopathological variables. Patients with a thyroglobulin level greater than 20 pmol/L had a significantly increased risk of disease recurrence on univariate analysis (n = 213 [P =.0001, log rank test]), and in the Cox proportional-hazards model, both advanced tumor stage (P =.001, relative hazard, 3.4 [95% confidence interval [CI]: 2.4-4.9]) and a thyroglobulin level greater than 20 pmol/L (P =.001, relative hazard, 5.1 [95% CI: 2.0-13.1]) were significant predictors of recurrence. No other variables significantly altered the hazards model. CONCLUSIONS: Advanced tumor stage at diagnosis and a stimulated thyroglobulin level greater than 20 pmol/L taken 3 months after total thyroideectomy was an independent predictor of disease recurrence. Patients with a thyroglobulin level greater than 20 pmol/L are at increased risk of recurrence and may be candidates for more intensive follow-up or additional treatment.


Clinical impact of (18)F-FDG PET in thyroid carcinoma patients with elevated thyroglobulin levels and negative (131)I scanning results after therapy.

Laking GR, Price PM.


Relationship between tumor burden and serum thyroglobulin level in patients with papillary and follicular thyroid carcinoma.

Bachelot A, Cailleux AF, Klain M, Baudin E, Ricard M, Bellon N, Caillou B, Travagli JP, Schlumberger M.

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Serum thyroglobulin (Tg) is a reliable marker for detecting recurrent and persistent disease during the follow-up of patients with papillary and follicular thyroid carcinoma. The goal of this study was to assess the relationship between the serum Tg level measured after thyroid hormone withdrawal and the tumor mass in thyroid cancer patients who underwent surgery with the use of an intraoperative probe for lymph node metastases with (131)I uptake. Patients were classified into one of three groups according to the Tg level: undetectable (n = 18); 1-10 ng/mL (n = 21); and greater than 10 ng/mL (n = 33). The main clinical characteristics and the extent of the disease at the time of initial treatment were similar in these three groups. Lymph node metastases were found in 13 of the 18 patients with undetectable Tg level. Eight patients had persistent foci of uptake after surgery that were located behind the sternoclavicular joint in six patients. The number of metastatic lymph nodes and their total surface (in mm(2)) or their total volume (in mm(3)) were significantly linked with serum Tg/thyrotropin [TSH] level (p = 0.002 and p < 0.0001, respectively). For a given metastatic surface
or volume, the serum Tg/TSH value was no longer linked with the number of metastatic lymph nodes (p = 0.32), suggesting that the total surface or total volume is the characteristic that best summarizes the influence of the disease on the serum Tg/TSH level. In conclusion, patients with higher serum Tg levels tend to have more extensive disease and should undergo more aggressive treatment modalities. Nevertheless, undetectable serum Tg should not be considered as a reliable criteria to exclude a minimal tumor burden in patients who have already been treated with (131)I.


Management of low-risk well-differentiated thyroid cancer based only on thyroglobulin measurement after recombinant human thyrotropin.

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A multicenter study was undertaken to ascertain prevalence and significance of recombinant human thyrotropin (rhTSH)-stimulated increases in thyroglobulin (Tg) levels in thyroid cancer patients classified to be at low risk for recurrence. Patients were eligible for enrollment if they had undergone near-total or total thyroidectomy and remnant ablation between 1-10 years prior to enrollment and had received thyroxine suppression therapy (THST) with a TSH level of < 0.5 mU/L and Tg level less than or equal to 5 ng/mL within the prior year. Patients with anti-Tg antibodies, distant metastases, or other evidence of residual disease were excluded. Four hundred eighty-six patients were entered into the study, and 300 were considered eligible and comprise the study population. TSH, Tg, and anti-Tg antibody levels were obtained at baseline, followed by intramuscular injection of 0.9 mg of rhTSH on days 1 and 2 and measurement of Tg on day 5. After rhTSH, 53 patients (18%) had elevations in Tg of at least 2 ng/mL, including 33 patients (11%) with increases from baseline of equal to or greater than 5 ng/mL. Patients with an initial advanced stage of disease were more likely to display elevations in Tg after rhTSH. One third of those with stage III disease displayed elevations in Tg of 2 ng/mL or more. Patients within 5 years of thyroidectomy were as likely to display elevations in rhTSH-stimulated Tg as those 5-10 years from surgery. In conclusion, these data suggest rhTSH-stimulated Tg testing without scan may be a useful tool in the follow-up of patients with low-risk thyroid cancer, and may serve to identify patients previously thought free of disease on the basis of undetectable Tg levels while undergoing THST. A strategy is presented for incorporation of this approach into the management of patients with low-risk well-differentiated thyroid cancer.
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Association of a thyroglobulin gene polymorphism with Hashimoto's thyroiditis in the Japanese population.

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Summary objective The aetiology of the autoimmune thyroid diseases (AITDs), Graves' disease (GD) and Hashimoto's thyroiditis is largely unknown. However, genetic susceptibility is believed to play a major role. Two whole genome scans from Japan and from the USA identified a locus on chromosome 8q24 which showed evidence for linkage with AITD and HT. Recent studies have demonstrated an association between a Tg polymorphism and AITD, suggesting that Tg is the susceptibility gene on 8q24. Patients We studied 308 Japanese AITD patients (194 GD and 114 HT patients) and 417 Japanese control subjects in association studies. Design Case-control association studies were performed using D8S284 and D8S272, microsatellite markers located in the 8q24 region, Tgms1 and Tgms2, microsatellite markers in introns 10 and 27, respectively, of Tg, and a SNP in exon 33 of Tg. Results No differences in allele frequencies were observed between AITD patients and controls for D8S284, D8S272 and Tgms1. Similarly, for Tgms2 and the exon 33 SNP no significant differences in allele frequency distribution were observed for all AITD patients. However, when analysing the HT patients alone we found a significant association between the 330 bp/352 bp genotype of Tgms2 and HT (HT = 16.7%, controls = 7.1%; corrected P-value = 0.01, OR = 2.6). Conclusion Our results confirm the previous reports of an association between the Tg gene and AITD and suggest that Tg is an AITD susceptibility gene.


Why measure thyroglobulin autoantibodies rather than thyroid peroxidase autoantibodies?

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Autoantibodies to thyroglobulin (TgAb) and thyroid peroxidase (TPOAb) are of immunoglobulin G (IgG) class and have high affinities for their respective autoantigens. Both autoantibodies are markers of thyroid autoimmunity and they can be measured by a variety of assays. From the clinical perspective, TgAb are less prevalent than TPOAb and less useful than TPOAb for prediction of thyroid dysfunction. Moreover, TgAb interfere with Tg measurements to monitor metastases in thyroid cancer. However, increasing evidence suggests that these TgAb provide a surrogate for Tg. In terms of disease pathogenesis, Tg has been suggested to play a role in Graves' ophthalmopathy. Pending further studies, TgAb epitopes could distinguish between individuals who are euthyroid or who have clinical disease. A final, intriguing reason for measuring and characterizing TgAb is the interest these autoantibodies have rekindled in their autoantigen. It is conceivable that Tg polymorphisms, combined with the explosive mix of iodine, TPO and H2O2 necessary for thyroid hormone synthesis, inadvertently provide the trigger for the autoimmune thyroid response.


The thyroglobulin gene as the first thyroid-specific susceptibility gene for autoimmune thyroid disease.

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Recent linkage and association studies provide evidence for thyroglobulin (Tg) being an autoimmune thyroid disease (AITD) susceptibility gene. The Tg locus has been reported to be linked with AITD in two independent studies, and further analysis demonstrated that markers within the Tg gene were associated with AITD. Furthermore, missense single-nucleotide polymorphisms (SNPs) in the Tg gene were shown to be associated with autoimmune thyroiditis in both mice and humans. If Tg is confirmed as a susceptibility gene for AITD, it could provide a novel therapeutic target.


The thyroglobulin gene: the third locus for autoimmune thyroid disease or a false dawn?

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Genetic studies have identified the HLA and CTLA4 regions as susceptibility loci for the development of common autoimmune thyroid diseases (AITDs), including Graves' disease and autoimmune hypothyroidism. Despite numerous studies, the identification of a third locus has remained elusive. Genetic-linkage studies have implicated chromosome 8q24 as a susceptibility locus for AITD. The gene encoding thyroglobulin (Tg), which encodes a major thyroid autoantigen, maps to this region, and a recent study has reported the association of several exonic single-nucleotide polymorphisms (SNPs) with disease. Although these preliminary data are potentially exciting, caution needs to be exercised, and replication of the data sought before Tg can be designated as the third locus for AITD.
**Molecular Biology & Immunology**

**Histone deacetylase inhibitors restore radioiodide uptake and retention in poorly differentiated and anaplastic thyroid cancer cells by expression of the sodium/iodide symporter thyroperoxidase and thyroglobulin.**


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Iodide uptake by the thyroid is mediated by the sodium/iodide symporter. Upon iodide uptake, thyroperoxidase catalyzes iodination of tyrosine residues in thyroglobulin, retaining iodide within thyroid follicles. Dedifferentiation-induced loss of these functions in cancers, rendering them unresponsive to radiodiode, occurs with most poorly differentiated and anaplastic tumors. We focused on the histone deacetylase (HDAC) inhibitors (HDACI) as a way to induce differentiation of thyroid cancer cells. We assessed re-expression of thyroid-specific genes mRNA induced by HDACI using quantitative RT-PCR and immunostaining in poorly differentiated papillary and anaplastic thyroid cancer cells. HDACI induced expression of thyroid-specific gene mRNAs and proteins, and accumulation of radioiodide through iodination of generic cellular proteins were detected. HDACI-treated tumors could specifically accumulate (125)I as revealed by imaging experiments and radioiodide concentration in vivo. In an attempt to determine the mechanism by which these gene expressions occurred, we detected the inhibition of protein synthesis by cycloheximide, which up-regulated the expression of thyroperoxidase and thyroglobulin mRNA in HDACI-treated cells and down-regulated that of sodium/iodide symporter mRNA. Together, our results suggest that HDACI-induced expression of thyroid-specific genes, some of which is mediated by some protein synthesis, may contribute to development of novel strategy against thyroid cancer.


**Antigenicity and immunogenicity of the C-terminal peptide of human thyroglobulin.**

El Hassani RA, Estienne V, Blanchin S, Durand-Gorde JM, Mallet B, De Micco C, Carayon P, Lalaoui K, Ruf J.

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Thyroglobulin (Tg) is cleaved into several peptides during thyroid hormone synthesis, an oxidative process. P40, an iodinated C-terminal peptide from human Tg, has a molecular weight of about 40 kDa and contains two hormonogenic sites. P40 is the smallest peptide that is still recognized by monoclonal antibodies from mice immunized with human Tg directed against its immunodominant region. Since P40 also contains several T-cell epitopes, it is a good candidate for studying the primary events involved in the process of hormone synthesis leading to thyroid autoimmunity. The present results show that P40 is recognized by Tg antibodies from patients with thyroid disorders and induces Tg antibodies in CBA mice. P40 may therefore be involved in the autoimmune process, thus providing a useful tool for diagnostic and therapeutic purposes.

**Immunology.** 2004 May;112(1):13-25.

**Thyroglobulin as an autoantigen: what can we learn about immunopathogenicity from the correlation of antigenic properties with protein structure?**

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Autoantibodies against human thyroglobulin are a hallmark of autoimmune thyroid disease in humans, and are often found in normal subjects. Their pathogenic significance is debated. Several B-cell epitope-bearing peptides have been identified in thyroglobulin. They are generally located away from the cysteine-rich regions of tandem sequence repetition. It is possible that our current epitopic map is incomplete because of the difficulty that proteolytic and recombinant approaches have in restituting conformational epitopes based upon proper pairing between numerous cysteiny1 residues. Furthermore, the homology of cysteine-rich repeats with a motif occurring in several proteins, endowed with antiprotease activity, suggests that these regions may normally escape processing and presentation to the immune system, and brings attention to the mechanisms, such as oxidative cleavage, by which such cryptic epitopes may be exposed. A number of T-cell epitope-bearing peptides, endowed with thyroditogenic power in susceptible mice, were also identified. None of them was dominant, as none was able to prime in vivo lymph node cells that would proliferate or transfer autoimmune thyroiditis to syngeneic hosts, upon stimulation with intact thyroglobulin in vitro. More than half of them are located within the acetylcholinesterase-homologous domain of thyroglobulin, and overlap B-cell epitopes associated with autoimmune thyroid disease, while the others are located within cysteine-rich repeats. The immunopathogenic, non-dominant character of these epitopes also favours the view that the development of autoimmune thyroid disease may involve the unmasking of cryptic epitopes, whose exposure may cause the breaking of peripheral tolerance to thyroglobulin. Further research in this direction seems warranted.


**The acetylcholinesterase homology region is essential for normal conformational maturation and secretion of thyroglobulin.**

Park YN, Arvan P.

Division of Metabolism, Endocrinology, and Diabetes and the Program of Cellular and Molecular Biology, University of Michigan Medical Center, Ann Arbor, Michigan 48109, USA.

Secretion of thyroglobulin (Tg, a large homodimeric glycoprotein) is essential to deliver Tg to its site of iodination for thyroxine biosynthesis. An L2263P missense mutation in Tg has been proposed as the molecular defect causing congenital goitrous

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Autoantibodies against human thyroglobulin are a hallmark of autoimmune thyroid disease in humans, and are often found in normal subjects. Their pathogenic significance is debated. Several B-cell epitope-bearing peptides have been identified in thyroglobulin. They are generally located away from the cysteine-rich regions of tandem sequence repetition. It is possible that our current epitopic map is incomplete because of the difficulty that proteolytic and recombinant approaches have in restituting conformational epitopes based upon proper pairing between numerous cysteiny1 residues. Furthermore, the homology of cysteine-rich repeats with a motif occurring in several proteins, endowed with antiprotease activity, suggests that these regions may normally escape processing and presentation to the immune system, and brings attention to the mechanisms, such as oxidative cleavage, by which such cryptic epitopes may be exposed. A number of T-cell epitope-bearing peptides, endowed with thyroditogenic power in susceptible mice, were also identified. None of them was dominant, as none was able to prime in vivo lymph node cells that would proliferate or transfer autoimmune thyroiditis to syngeneic hosts, upon stimulation with intact thyroglobulin in vitro. More than half of them are located within the acetylcholinesterase-homologous domain of thyroglobulin, and overlap B-cell epitopes associated with autoimmune thyroid disease, while the others are located within cysteine-rich repeats. The immunopathogenic, non-dominant character of these epitopes also favours the view that the development of autoimmune thyroid disease may involve the unmasking of cryptic epitopes, whose exposure may cause the breaking of peripheral tolerance to thyroglobulin. Further research in this direction seems warranted.


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Division of Metabolism, Endocrinology, and Diabetes and the Program of Cellular and Molecular Biology, University of Michigan Medical Center, Ann Arbor, Michigan 48109, USA.

Secretion of thyroglobulin (Tg, a large homodimeric glycoprotein) is essential to deliver Tg to its site of iodination for thyroxine biosynthesis. An L2263P missense mutation in Tg has been proposed as the molecular defect causing congenital goitrous
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hypothyroidism in cog/cog mice due to perturbed Tg homodimerization, resulting in its retention within the endoplasmic reticulum. The mutation falls within a carboxyl-terminal region of Tg with high structural similarity to the entirety of acetylcholinesterase-like (AChE), a secretary protein that also forms homodimers. We provide new evidence that authentic AChE and the cholinesterase-like domain of Tg share a common tertiary structure. Moreover, we find that a Tg truncation, deleted of the cholinesterase-like region (but not a comparably sized deletion of internal Tg regions), blocks Tg export. Appending to this truncation a cDNA encoding authentic AChE results in translation of a chimeric protein in which AChE is present in a native, enzymatically active (albeit latent) conformation, and this fully rescues Tg secretion. Introduction of the cog mutation inhibits AChE enzyme activity, and established denaturing mutations of AChE block secretion of the Tg. Additional studies show that the native structure of the AChE region functions as a "dimerization domain," facilitating intracellular transport of Tg to the site of thyroid hormoneogenesis.


Risk of non-mediterranean thyroid cancer influenced by polymorphic variation in the thyroglobulin gene.


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Benign thyroid disorders are strong risk factors for non-mediterranean thyroid cancer (NMTC). Germline variation in Tg (thyroglobulin) and TSHR (thyroid stimulating hormone receptor) confers an increased risk of benign thyroid disorders. To explore the hypothesis that polymorphic variation in these genes affects the risk of NMTC we compared the frequency of TgQ2511R, TSHR-P52T and TSHR-D727E genotypes in two series of NMTC cases and controls (group 1, Canadian 102 cases and 102 controls; group 2, British 202 cases and 298 controls). No significant association was seen with TSHR-P52T and TSHR-D727E genotypes and risk of NMTC. However, the frequency of the R-allele of TgQ2511R was over represented in NMTC cases in both study populations. The odds ratios associated with hetero- and homozygosity for the R-allele were 1.6 (95% confidence interval, 1.1-2.5) and 2.0 (95% confidence interval, 1.2-3.3), respectively. Although the risk of NMTC associated with the TgQ2511R R-allele is modest, its high prevalence in the general population suggests it may make a significant contribution to the incidence of NMTC.

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Two distinct compound heterozygous constellations (R277X/IVS34-1G>C and R277X/R1511X) in the thyroglobulin (TG) gene in affected individuals of a Brazilian kindred with congenital goiter and defective TG synthesis.

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In this study, we have extended our initial molecular studies of a nonconsanguineous family with two affected siblings and one of their nephews with congenital goiter, hypothyroidism, and marked impairment of thyroglobulin synthesis. Genomic DNA sequencing revealed that the index patient (affected nephew) was heterozygous for a single base change of a cytosine to a thymine at nucleotide 886 in exon 7 (886C>T, mother's mutation) in one allele and for a novel guanine to cytosine transversion at position -1 of the splice acceptor site in intron 34 (IVS34-1G>C, father's mutation) in the other allele. The two affected siblings inherited the 886C>T mutation from their mother and a previously reported cytosine to thymine transition at nucleotide 4588 in exon 22 from their father (4588C>T). The 886C>T and 4588C>T substitutions resulted in premature stop codons at amino acids 277 (R277X) and 1511 (R1511X), respectively. In vitro transcription analysis showed that the exon 35 is skipped entirely when the IVS34-1G>C mutation is present, whereas the wild-type allele is correctly spliced. SSCP (exon 7 and 35) and restriction analysis (exon 22) using Taq I indicated that the two affected siblings, the affected nephew, his mother, and his unaffected brother were all heterozygous for the R277X mutation. The two affected siblings, their father, and three unaffected siblings were all heterozygous for the IVS34-1G>C mutation. Moreover, in this kindred, we have characterized polymorphisms (insertion/deletion, microsatellite, and single nucleotide polymorphism) located within introns 18 and 29 and exon 44 that are associated with the described mutations. Haplotype analysis with these polymorphic markers in two unrelated Brazilian families (present family studied and previously reported family) harboring the R277X mutation suggests a founder effect for the R277X mutation. In conclusion, the affected individuals of this family are either compound heterozygous for R277X/IVS34-1G>C or R277X/R1511X. This observation further supports that thyroglobulin gene mutations display significant intraallelic heterogeneity.


Evidence for processing of compact insoluble thyroglobulin globules in relation with follicular cell functional activity in the human and the mouse thyroid.

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OBJECTIVE: Thyroglobulin (Tg) is stored within the follicular lumen mainly in a soluble form, but globules made of insoluble multimers are also present and considered to be a mechanism to store prohormone at high concentration. We investigated the immunohistochemical properties of these intrafollicular globules and their possible processing by thyroid cells upon stimulation in the human and in the mouse. DESIGN: Human thyroids (normal, Graves' disease and hot adenomas) and thyroids from old ICR mice without or with goitrogenic treatment were processed for light microscopy. METHODS: Immunohistochemistry for Tg with a polyclonal antibody and two monoclonal antibodies, one specific for thyroxine-rich-iodinated Tg and the other recognizing Tg independently of its iodine level, staining with periodic-acid-schiff, and binding of lectins specific for mannose and sialic acid were performed on all tissue sections. Intrafollicular globules were quantified, with distinction between 'active' or 'hot' and 'hypofunctioning' or 'cold' follicles. RESULTS: In normal human and old mouse thyroids, the intrafollicular globules were strongly stained with PAS, but
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negative for the three anti-Tg antibodies and the two lectin-binding assays, while the surrounding soluble Tg was positive. In normal human tissue, globules were more frequent in 'hypofunctioning' than in 'active' follicles. They were exceptional in Graves' disease and hot adenomas. In old mice, Tg globules were more frequent in 'cold' than in 'hot' follicles. Along with the goitrogen treatment, they became fewer, fragmented and more often present in follicles with a 'hot' aspect. CONCLUSIONS: Upon TSH stimulation, thyrocytes become able to process colloid globules suggesting that this stock of Tg can be used in vivo for thyroid hormone synthesis.


Thyroglobulin-pulsed human monocyte-derived dendritic cells induce CD4+ T cell activation.


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Although thyroglobulin (Tg) would be expected to act as a tumor-associated antigen that might be exploitable by immunotherapy against thyroid cancers, it remains unclear how to effectively enhance the immune response to Tg in human since it is a self-component glycoprotein. We therefore tested whether and how human peripheral blood (PB) monocyte-derived dendritic cells (DCs) pulsed with human (h)Tg would induce activation of hTg-specific T cells. We found that immature DCs (iDCs) exhibited a higher endocytic capacity for fluorescein isothiocyanate-conjugated hTg than did mature DCs (mDCs). Although freshly isolated T cells responded poorly to mDCs, hTg-primed T cells responded much more strongly to hTg pulsed mDCs, which selectively induced IFN-gamma-secreting T cells. These results suggest that hTg-pulsed mDCs enhance the responses of Tg-specific T cells, raising the possibility that vaccination with hTg-pulsed mDCs may be an effective approach as immunotherapy to potentiate thyroid cancer specific therapy.


Preferential megalin-mediated transcytosis of low-hormonogenic thyroglobulin: a control mechanism for thyroid hormone release.


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Hormone secretion by thyrocytes occurs by fluid phase uptake and lysosomal degradation of the prohormone thyroglobulin (Tg). However, some Tg internalized by megalin bypasses lysosomes and is transcytosed across cells and released into the bloodstream. Because the hormone content of Tg is variable, we investigated whether this affects transcytosis. We found that rat Tg with a low hormone content [low-hormonogenic rat Tg (low-horm-rTg)] is transcytosed by megalin across thyroid FRTL-5 cells to a greater extent than rat Tg with a high hormone content [hormonogenic rat Tg (horm-rTg)]. In immunoprecipitation experiments, the Tg sequence Arg-2489-Lys-2503 (required for binding to megalin and heparan sulfate proteoglycans) was found to be more exposed in low-horm-rTg, which accounted for its preferential transcytosis. Thus, removal of surface heparan sulfate proteoglycans from FRTL-5 cells or blocking of 2489-2503 reduced transcytosis of low-horm-rTg to a greater extent than that of horm-rTg. Preferential transcytosis of low-horm-rTg affected hormone release. Thus, the increase in hormone release from horm-rTg in FRTL-5 cells determined by megalin blocking (due to reduced transcytosis and enhanced Tg degradation) was rescued by low-horm-rTg, suggesting that megalin is required for effective hormone release. This finding was confirmed in a small number of megalin-deficient mice, which had serological features resembling mild hypothyroidism. Reduced hormone formation within Tg in vivo, due to treatment of rats with aminothiazole or of patients with Graves' disease with methimazole, resulted in increased Tg transcytosis via megalin, in confirmation of results with FRTL-5 cells. Our study points to a major role of megalin in thyroid homeostasis with possible implications in thyroid diseases.


Amino acid substitutions in the thyroglobulin gene are associated with susceptibility to human and murine autoimmune thyroid disease.

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The 8q24 locus, which contains the thyroglobulin (Tg) gene, was previously shown to be strongly linked with autoimmune thyroid disease (AITD). We sequenced all 48 exons of the Tg gene and identified 14 single-nucleotide polymorphisms (SNPs). Case control association studies demonstrated that an exon 10-12 SNP cluster and an exon 33 SNP were significantly associated with AITD (P < 0.01). Haplotype analysis demonstrated that the combination of these two SNP groups was more significantly associated with AITD (P < 0.001). Gene-gene interaction studies provided evidence for an interaction between HLA-DR3 and the exon 33 SNP, giving an odds ratio of 6.1 for Graves' disease. We then sequenced exons 10, 12, and 33 of the mouse Tg gene in 19 strains of mice. Fifty percent of the strains susceptible to thyroiditis had a unique SNP haplotype at exons 10 and 12, whereas none of the mouse strains that were resistant to thyroiditis had this SNP haplotype (P = 0.01). We concluded that Tg is a susceptibility gene for AITD, both in humans in and in mice. A combination of at least two Tg SNPs conferred susceptibility to human AITD. Moreover, the exon 33 SNP showed evidence for interaction with HLA-DR3 in conferring susceptibility to Graves’ disease.
Thyrotropin and iodide regulate sulfate concentration in thyroid cells. Relationship to thyroglobulin sulfation.

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Thyroglobulin (Tg), the thyroid hormone precursor, is sulfated on tyrosines and on carbohydrates. We showed recently that sulfated tyrosines were involved in thyroid hormone synthesis. Moreover, we also reported that Tg sulfation is downregulated by thyrotropin (TSH), especially on tyrosines. This control may occur at each step in the sulfation process. In this paper, we studied the regulation of the concentration of cytosolic inorganic sulfate, the first substrate, in porcine thyroid cells stimulated by TSH with or without iodide. The amounts of cytosolic sulfate and the cytosolic volumes measured showed that the sulfate concentration depends only on cytosolic volume changes in response to TSH and iodide treatment. After the cells were labelled with [35S]-sulfate, the specific radioactivity (SRA) of cytosolic sulfate was determined. When cells were treated with only TSH, the concentration and SRA of cytosolic sulfate decreased by 30%, and by about 15% when cells were incubated with both TSH and iodide. TSH decreased more conspicuously the rate of [35S]-sulfate incorporation into Tg (by 57% without iodide, by 43% with iodide) than the concentration and SRA of cytosolic sulfate, while iodide altered these parameters to the same extent (15%). These findings suggest that TSH regulates other steps in the sulfation process, such as specific substrate and enzyme levels, while iodide controls mainly the sulfate concentration.

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Secretion of thyroglobulin (Tg) by thyrocytes requires several endoplasmic reticulum (ER)-resident molecular chaperones. The receptor-associated protein (RAP), a known molecular chaperone, binds to Tg in thyroid cells shortly after biosynthesis. Here we investigated whether RAP is involved in Tg secretion by FRTL-5 cells. For this purpose, we studied Tg secretion by FRTL-5 cells transfected with a soluble RAP chimera, as a mean for interfering with endogenous RAP. We used a RAP-human IgG Fc (RAP-Ig) chimeric cDNA, which was designed in order to exclude the ER retention sequence of RAP and to allow generation of a secreted form of RAP. FRTL-5 cells were transiently transfected with the RAP-Ig cDNA or, as control, with a CD8-Ig cDNA. Media were collected at 24, 48 and 72 h after transfection. Secretion of fusion proteins and of Tg in the media was measured by ELISA. As expected, under standard culture conditions, RAP was not secreted into the media by FRTL-5 cells, even though it could be detected by Western blotting in cell extracts. In transfection experiments, fusion proteins were present in the media of FRTL-5 cells transfected with either RAP-Ig or CD8-Ig, indicating that transfection was successful. Although Tg was found in the media of FRTL-5 cells transfected with either CD8-Ig or RAP-Ig, a lower amount was found in cells transfected with RAP-Ig. Therefore, we concluded that RAP is involved in Tg secretion by FRTL-5 cells suggesting that RAP may function as a Tg molecular chaperone.


The RHL-1 subunit of the asialoglycoprotein receptor of thyroid cells: cellular localization and its role in thyroglobulin endocytosis.


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The rat hepatic lectin (RHL-1) is the major component of the rat liver asialoglycoprotein receptor (ASGPr), a membrane receptor highly expressed on the basolateral side of hepatocytes, which mediates endocytosis of serum desialated glycoproteins. We have recently shown that RHL-1 is expressed in rat thyroid tissue and thyroid differentiated cell lines. Both in vitro and in vivo assays show that thyrotropin up-regulates thyroid RHL-1 expression, while neoplastic transformation of thyroid cells exerts a down-regulation of receptor expression. Moreover, RHL-1 expressed on the surface of differentiated thyroid cells is able to bind thyroglobulin (Tg), the macromolecular site of synthesis and storage of thyroid hormones. In the present work, we demonstrate, by immunohistochemistry, that RHL-1 is localized on the apical surface of thyrocytes, at a variance with its basolateral localization on hepatocytes. Moreover, albeit its expression in thyroid is less abundant than in liver, the receptor is able to bind asialorosomucoid (ASOR), the best-known ligand of hepatic ASGPr, and to mediate endocytosis of a significative amount of Tg on the surface of differentiated PC13 thyroid cells. Taken together, the data suggest that RHL-1, even if expressed in thyroid at lower levels than in liver, could serve as a receptor for endocytosis of colloidal Tg and, likely, for its delivery to lysosomes.


Association of a rare thyroglobulin gene microsatellite variant with autoimmune thyroid disease.

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Genetic and environmental factors contribute to the development of Graves’ disease and Hashimoto’s thyroiditis. These diseases, although clinically distinct, share many immunological and histological features. Susceptibility genes for autoimmune thyroid disease (AITD) have been investigated, although only the human leukocyte antigen and cytotoxic T lymphocyte-associated antigen-4 gene...
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regions have been consistently associated with disease. Recent data, however, have shown linkage and association of chromosome 8q24 (containing the thyroglobulin gene) to AITD. Therefore, we performed a case-control association study on patients with AITD and controls using previously associated markers (D8S284 and Tgms2). No differences in allele frequencies were observed between AITD cases and controls for D8S284. Compared with the three common alleles (frequencies >10%), the rare alleles of Tgms2 were increased (chi(2)= 10.6; P = 0.001) at Tgms2. This group included the 336-bp allele (increased in cases vs. controls: chi(2)= 24.97; P < 0.001), which has previously been reported to be associated with AITD. The rarity of this allele in the United Kingdom, however, precluded analysis in our family dataset. Although these findings may represent a random chance event, in view of previous reports of linkage and association of this gene region to AITD, this may be an example of a rare causal variant of a complex disease.  


Correlation between the loss of thyroglobulin iodination and the expression of thyroid-specific proteins involved in iodine metabolism in thyroid carcinomas.


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Progress in biotechnology has provided useful tools for tracing proteins involved in thyroid hormone synthesis in vivo. Mono- or polyclonal antibodies are now available to detect on histological sections the Na(+)/I(-) symporter (NIS) at the basolateral pole of the cell, the putative iodide channel (pendrin) at the apical plasma membrane, thyroperoxidase (TPO), and members of the NADPH-oxidase family, thyroid oxidase 1 and 2 (ThOXs), part of the H(2)O(2)-generating system. The aim of this study was to correlate thyroglobulin (Tg) iodination with the presence of these proteins. Tg, T(4)-containing Tg, NIS, pendrin, TPO, ThOXs, and TSH receptor (TSHr) were detected by immunohistochemistry on tissue sections of normal thyroids and various benign and malignant thyroid disorders. Tg was present in all cases. T(4)-containing Tg was found in the adenomas, except in Hurthle cell adenomas. It was never detected in carcinomas. NIS was reduced in all types of carcinomas, whereas it was detected in noncancerous tissues. Pendrin was not expressed in carcinomas, except in follicular carcinomas, where weak staining persisted. TPO expression was present in insular, follicular carcinomas and in follicular variants of papillary carcinomas, but in a reduced percentage of cells. It was below the level of detection in papillary carcinomas. The H(2)O(2)-generating system, ThOXs, was found in all carcinomas and was even increased in papillary carcinomas. Its staining was apical in normal thyroids, whereas it was cytoplasmic in carcinomas. The TSHr was expressed in all cases, but the intensity of the staining was decreased in insular carcinomas. In conclusion, our work shows that all types of carcinomas lose the capacity to synthesize T(4)-rich, iodinated Tg. In follicular carcinomas, this might be due to a defect in iodide transport at the basolateral pole of the cell. In papillary carcinomas, this defect seems to be coupled to an altered apical transport of iodide and probably TPO activity. The TSHr persists in virtually all cases.

The cryptic self in thyroid autoimmunity: the paradigm of thyroglobulin.

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Recent studies have increased the number of known thyroiditogenic sites in thyroglobulin (Tg) to thirteen. These sites contain T-cell epitopes and are scattered throughout Tg, with nine of them localized toward the carboxyl terminal third of the molecule. So far, no pathogenic determinant has been found to be dominant, i.e. to be readily and consistently generated in extrathyroidal antigen-presenting cells (APC) following processing of intact Tg in vivo and in vitro. However, certain conditions, such as internalization of Tg-antibody complexes or enhanced iodination of Tg, have been described to promote generation of cryptic pathogenic peptides in APC, in vitro. These findings support the view that post-translational events can "unmask the cryptic self" and suggest mechanisms that may contribute to the pathogenesis of thyroiditis.

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Glycosaminoglycans provide a binding site for thyroglobulin in orbital tissues of patients with thyroid-associated ophthalmopathy.


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The presence of thyroglobulin (Tg) in orbital tissues of patients with thyroid-associated ophthalmopathy (TAO) supports a role of Tg in TAO pathogenesis. To search for Tg-binding sites in orbital tissues, because Tg is a heparin-binding protein, we investigated its binding to glycosaminoglycans (GAGs) that are abundant in orbital tissues; chondroitin sulfate B (CSB) and C (CSC) and hyaluronic acid (HA). Both in solid phase and solution phase assays purified human Tg bound to GAGs. In solid-phase assays, binding was increased by coinubation with heparin or GAGs in solution, or with an antibody against a Tg heparin-binding sequence (Arg2489-Glu2503), possibly suggesting crosslinking of Tg molecules induced by GAGs or by the presumably bivalent antibody. Orbital tissue extracts from TAO patients that contained Tg were subjected to high-salt treatment, which resulted in separation of Tg from GAGs, as observed by column chromatography. After separation from GAGs, the Tg in orbital tissue extracts acquired the ability to bind to immobilized CSB, and heparin enhanced binding, resembling the findings with purified human Tg. Therefore, we conclude that GAGs provide binding sites for Tg in orbital tissues, which may explain the presence of Tg in orbital tissues of patients with TAO.
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Thyroglobulin epitope recognition in a post iodine-supplemented Sri Lankan population.


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OBJECTIVE: We previously reported a high prevalence of raised thyroglobulin autoantibodies (TgAb) in apparently healthy Sri Lankan schoolgirls following salt iodination. To characterize these antibodies further we determined the epitopes on thyroglobulin (Tg) with which they react and compared these with serum obtained from both healthy subjects and established autoimmune thyroid disease (AITD) patients from the UK. To extend our study to a wider population within Sri Lanka, we in addition determined the epitopes recognized by a group of AITD patients selected from a thyroid clinic in Sri Lanka, as well as apparently healthy female Sri Lankan tea workers of distinct ethnicity from the schoolgirls and AITD patients. DESIGN: Sri Lankan schoolgirls (n = 282) and adult female tea estate workers (n = 208) were examined for thyroid autoimmunity markers. Sera with high TgAb (> 98 kIU/l) were selected from these two groups (n = 36 and 45, respectively) to study epitope-binding patterns. We also examined the sera from 16 AITD patients attending a thyroid clinic in Colombo, 16 patients with AITD from the thyroid clinic at the University Hospital of Wales and 16 sera from healthy control UK women with no evidence of thyroid disease. To determine the epitopes on Tg recognized by the subjects' TgAb, we employed a panel of Tg mouse monoclonal antibodies labelled with alkaline phosphatase in a competitive enzyme-linked immunosorbent assay reaction with the subjects' serum. RESULTS AND CONCLUSIONS: A majority of the Sri Lankan schoolgirls did not react with the immunodominant epitopes and did not differ significantly from healthy subjects from the UK in their Tg epitope recognition pattern. On the other hand, tea estate workers and Sri Lankan AITD patients recognized typical autoimmune thyroid disease epitopes and, in addition, recognized a separate cluster not previously associated with either the autoimmune state or the healthy state. The significance of this cluster requires further clarification.


Compound heterozygous mutations in the thyroglobulin gene (1143delC and 6725G-->A [R2223H]) resulting in fetal goitrous hypothyroidism.

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In a 22-year-old healthy woman, a fetal goiter was diagnosed coincidentally by ultrasound during the sixth month of gestation, and hypothyroidism was affirmed by a high TSH (336 mU/liter) concentration after cordocentesis. A second ultrasound examination at 27 wk gestation showed further enlargement of the goiter (34/21 mm). Two intraamniotic injections of 200 microg levothyroxine were performed during the seventh month of pregnancy. Ultrasound studies revealed a fetal goiter size of 30/18 mm during the eighth month of gestation. The woman delivered at term a female infant with an Apgar score of 10 at 1 and 5 min. Cord blood analysis indicated elevated TSH (284 mU/liter) and low free T(4) (5.5 pmol/liter) levels. The serum thyroglobulin (Tg) concentration was low (0.8 ng/ml), whereas ultrasound of the neonate indicated an enlarged thyroid gland (32/15/14 mm). During the second pregnancy, ultrasound examination revealed a goiter, and fetal hypothyroidism was also confirmed after umbilical vein blood sampling (TSH, 472 mU/liter). After two intraamniotic injections of 500 microg levothyroxine, the woman delivered a male infant at 37 wk of pregnancy. In cord blood the serum TSH concentration was 39 mU/liter, and the serum Tg level was low (0.7 ng/ml). The parents were nonconsanguineous. After birth of the two affected siblings, genomic DNA sequencing identified the presence of compound heterozygous mutations of the Tg gene: the paternal mutation consists of a cytosine deletion at nucleotide 1143 in exon 9 (1143delC), resulting in a frameshift that generates a stop codon at position 382, and the maternal mutation is a guanine to adenine substitution at position 6725 in exon 38, creating the R2223H missense mutation in the acetylcholinesterase homology domain of Tg. In conclusion, we report two siblings with congenital goiter and hypothyroidism caused by compound heterozygous mutations of the Tg gene.


Proteomic and postproteomic characterization of keratansulfate-glycanated isoforms of thyroglobulin and transferrin uniquely elaborated by papillary thyroid carcinomas.


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Previous studies have suggested that surface components of papillary thyroid carcinoma (PTC) cells may be aberrantly glycanated, but the precise nature of these molecules has not been unveiled nor documented to be of clinical relevance. A monoclonal antibody was raised against a unique keratan sulfate (KS) determinant and used to differentially screen benign and malignant thyroid tissue for the expression of components carrying these moieties. In a total of 349 cases of benign and malignant thyroid lesions, 100% of the 115 PTC cases examined (including various histological subtypes) were found to contain KS-bearing molecules, whereas these were virtually absent from benign tissues and other thyroid tumors, with the exception of 21% of the follicular carcinoma cases analyzed. A composite immunoaffinity chromatography, immunochromatography, and mass spectrometric approach revealed that the PTC-specific KS-bearing macromolecules were unique glycoforms of thyroglobulin and transferrin. Combined, reciprocal immunoprecipitation and Western blotting further indicated that the former glycoform predominated and that most of the transferrin produced by PTC was glycanated with KS moieties. Fluorescent keratanase II-based fingerprinting of the KS moieties bound to these isoforms further demonstrated several PTC-specific peculiarities: 1) that a considerable portion of the moieties was covalently attached via a novel core protein linkage structure; 2) they had an unusual extended average length; 3) an unusual relative ratio of highly sulfated disaccharides terminating with alpha (2-3)-linked N-acetylatedamamic acid capping residues; and 4) a novel unidentified oligosaccharide moiety at the nonreducing terminus. Comparative analysis of the relative distribution of transferrin in benign versus PTC tissues highlighted a marked malignancy-
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associated abundance of the molecule, with a >75% frequency in expression in PTC. These findings demonstrate that PTC cells synthesize unique post-translationally modified thyroglobulin and transferrin variants in situ that may be directly exploitable for diagnosis, through histological and noninvasive cytological procedures; for devising novel strategies for antibody-guided imaging of this tumor in vivo; and for post-surgery follow-up of PTC patients.


Evidence for intramolecular B-cell epitope spreading during experimental immunization with an immunogenic thyroglobulin peptide.

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Thyroglobulin (Tg) is a target autoantigen in autoimmune thyroid diseases, such as Graves’ disease (GD) and Hashimoto’s thyroiditis. In a previous study we identified three 20mer Tg peptides bearing epitopes of autoantibodies associated with GD (TgP15, TgP26 and TgP41: sequences 2339-2358, 2471-2490 and 2651-2670 of human Tg, respectively). In the present study, we investigated the antigenicity of the above peptides in experimental immunization with Tg, the immunogenicity of antigenic peptides and the possibility of intramolecular-B-cell epitope spreading during peptide immunization. For this purpose, two rabbits were injected with human Tg in CFA six times, every three weeks. Two control animals were injected only with CFA. Testing of antisera and of affinity-purified antibodies, by ELISA against the three peptides, revealed reactivity only to TgP41. This synthetic peptide was subsequently administered to two rabbits, in its free form (100 micro g in CFA six times, every two weeks). A strong serological response was developed not only against TgP41, but also to intact human and rabbit Tg. Immunization with TgP41 induced intramolecular-B-cell epitope spreading, i.e. production of antibodies to sites on Tg other than that corresponding to TgP41, as revealed by immunoadsorption and competitive ELISA. Histopathological studies did not reveal any infiltration in thyroid glands. We conclude that peptide TgP41 encompasses not only an epitope of disease-associated autoantibodies, but also a dominant immunogenic epitope of experimentally induced Tg-specific antibodies, able to drive B-cell epitope spreading.


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Fms-like tyrosine kinase receptor 3-ligand (Flt3-L) and GM-CSF cause expansion of different subsets of dendritic cells and skew the immune response toward predominantly Th1 and Th2 type, respectively. In the present study, we investigated their effects on experimental autoimmune thyroiditis in CBA/J mice. Relative to mouse thyroglobulin (mTg) immunized controls, mTg-immunized mice treated with Flt3-L showed more severe thyroiditis characterized by enhanced lymphocytic infiltration of the thyroid, and IFN-gamma and IL-2 production. In contrast, mice treated with GM-CSF, either before or after immunization with mTg, showed suppressed Th cell response to mTg and failed to develop thyroiditis. Lymphocytes from these mice, upon activation with mTg in vitro, produced higher levels of IL-4 and IL-10. Additionally, GM-CSF-treated mice showed an increase in the frequency of CD4(/+)CD25(/+) T cells, which suppressed the mTg-specific Th cell response. Neutralization of IL-10, but not IL-4, or depletion of CD4(/+)CD25(/+) cells resulted in increased mTg-specific in vitro Th cell proliferation suggesting that IL-10 produced by the Ag-specific CD4(/+)CD25(/+) regulatory T cells might be critical for disease suppression. These results indicate that skewing immune response toward Th2, through selective activation of dendritic cells using GM-CSF, may have therapeutically potential in Th1 dominant autoimmune diseases including Hashimoto’s thyroiditis.


The pathogenic role of anti-thyroglobulin antibody on pregnancy: evidence from an active immunization model in mice.


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BACKGROUND: The presence of antibodies to thyroglobulin (Tg) is associated with fetal loss even in the absence of thyroid dysfunction. The aim of this study was to examine whether active immunization with Tg could elicit anti-Tg autoantibodies and reproductive failure without interfering with thyroid function. METHODS: BALB/c mice that were immunized with human Tg in complete Freund’s adjuvant (CFA) or injected with only CFA were studied for the development of antibodies to Tg, T4, dsDNA, ssDNA and cardiolipin. Total T4, free T4 and thyroid-stimulating hormone (TSH) levels were also assessed before and during pregnancy. Percentages of resorbed fetuses (the equivalent to human missed abortion) were compared and autoantibody presence on the placenta and fetuses was examined. RESULTS: Following immunization, high levels of anti-Tg were observed in mice immunized with Tg, compared with mice injected with CFA [0.83 +/- 0.23 versus 0.012 +/- 0.016 respectively; mean +/- SD optical density (OD) at 405 nm; P < 0.001]. The specificity of binding to Tg was confirmed by competition assay. Although total T4 levels were increased in comparison with control mice, this was associated with the presence of antibodies to T4. Indeed, free T4 levels and TSH were similar to control mice. Mice were killed after 14 days of pregnancy. The thyroid function and the histology of the thyroid glands were normal. Increased fetal wastage was found among the Tg-immunized mice compared with the CFA-injected mice (P = 0.04), with lower fetal and placental weights (fetal weights: 194 +/- 4 mg versus 240 +/- 6 mg; placental weights: 105 +/- 2 mg versus 130 +/- 3; P < 0.001 for
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both). Antibodies to Tg were demonstrated only on the placenta of Tg-immunized mice. CONCLUSION: Immunization with Tg results in the production of Tg antibodies and fetal resorption. These effects occur in the absence of thyroid dysfunction.


Induction of murine thyroiditis by a non dominant E(k)-restricted peptide of human thyroglobulin.

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We have previously shown that the human thyroglobulin (hTg) 20-mer peptide p2340 (aa 2340-2359) contains an epitope recognized by Tg-reactive B cells in patients with Graves' disease. The presence of several Ek-binding motifs within p2340 prompted us to examine whether this peptide can stimulate a T-cell response and elicit experimental autoimmune thyroiditis (EAT) in AKR/J (H-2k) mice. The peptide was found to be immunogenic at the T-cell level since it induced specific proliferative responses as well as interleukin-2 and interferon-gamma secretion in secondary cultures of peptide-primed lymph node cells (LNC). The p2340-specific proliferation was blocked almost completely by an Ek-specific monoclonal antibody (mAb) but was unaffected by a control Ak-specific mAb. Peptide-primed LNC did not respond to intact hTg and conversely, LNC primed in vivo with hTg did not respond to p2340 in culture, suggesting that p2340 contains non-dominant T-cell epitope(s). Direct subcutaneous challenge of AKR/J mice (n = 9) with p2340 in adjuvant, elicited mild to moderate EAT (infiltration index of 1-2) and strong p2340-specific immunoglobulin G responses in all mice tested. These data delineate a new thyroiditogenic sequence within the carboxyl terminal region of hTg.

Identification and characterization of a novel large insertion/deletion polymorphism of 1464 base pair in the human thyroglobulin gene.

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We identified a novel large insertion/deletion (Indel) polymorphism of 1464 bp localized in intron 18 of the human thyroglobulin gene. Data from sequence showed a high A+T content (62%), two 17-bp long motif repeats, and three different types of 10-bp long palindromic sequences. The comparison between these 1464 bp and sequences deposited in National Center for Biotechnology Information (NCBI)/GenBank database exhibit a nonsignificant degree of homology with any previously described sequences. The long polymerase chain reaction (PCR) method was used to amplify the genomic DNA region containing intron 17/exon 18/intron 18/exon 19/intron 19 by primers situated in the introns 17 and 19. The amplification generates two fragments of 3.5 and 5.0 kb that correspond to the exclusion or inclusion of a 1464-bp segment, respectively. Both variants are thus widely represented in the human population; giving allele frequencies of 0.56 (insertion) and 0.44 (deletion). Finally, the polymorphism was confirmed by sequence analysis of the 5.0- and 3.5-kb amplified fragments.


Folding of thyroglobulin in the calnexin/calreticulin pathway and its alteration by loss of Ca2+ from the endoplasmic reticulum.


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During its initial folding in the endoplasmic reticulum (ER), newly synthesized thyroglobulin (Tg) is known to interact with calnexin and other ER molecular chaperones, but its interaction with calreticulin has not been examined previously. In the present study, we have investigated the interactions of endogenous Tg with calreticulin and with several other ER chaperones. We find that, in FRTL-5 and PC-C13 cells, calnexin and calreticulin interact with newly synthesized Tg in a carbohydrate-dependent manner, with largely overlapping kinetics that are concomitant with the maturation of Tg intrachain disulphide bonds, preceding Tg dimerization and exit from the ER. Calreticulin co-precipitates more newly synthesized Tg than does calnexin; however, using two different experimental approaches, calnexin and calreticulin were found in ternary complexes with Tg, making this the first endogenous protein reported in ternary complexes with calnexin and calreticulin in the ER of live cells. Depletion of Ca(2+) from the ER elicited by thapsigargin (a specific inhibitor of ER Ca(2+)-ATPases) results in retention of Tg in this organelle. Interestingly, thapsigargin treatment induces the premature exit of Tg from the calnexin/calreticulin cycle, while stabilizing and prolonging interactions of Tg with BiP (immunoglobulin heavy chain binding protein) and GRP94 (glucose-regulated protein 94), two chaperones whose binding is not carbohydrate-dependent. Our results suggest that calnexin and calreticulin, acting in ternary complexes with a large glycoprotein substrate such as Tg, might be engaged in the folding of distinct domains, and indicate that luminal Ca(2+) strongly influences the folding of exportable glycoproteins, in part by regulating the balance of substrate binding to different molecular chaperone systems within the ER.
Molecular Biology & Immunology


Targeting of thyroglobulin to transcytosis following megalin-mediated endocytosis: evidence for a preferential pH-independent pathway.

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TG internalized from the colloid by megalin, bypasses the lysosomal pathway and is transported across thyocytes by transcytosis. Although most of the intracellular mechanisms responsible for targeting of ligands to transcytosis are unknown, for certain ligands a role of lysosomal pH has been established. Thus, ligands that undergo lysosomal degradation dissociate from their receptors due to the low pH of endosomes, whereas certain ligands that undergo transcytosis fail to dissociate because they bind to their receptors at acidic pH. Here we studied the role of pH in TG transcytosis. We first investigated the effect of pH on megalin binding to TG in solid phase assays and found that, although megalin bound to TG at various pH values (ranging from 4-8), optimal binding was seen at acidic pH (ranging from 4.5-6). We then studied the effect of chloroquine (CQ) and ammonium chloride (AC), which increase endosomal pH, on transcytosis of TG across Fisher rat thyroid (FRTL-5) cells. Transcytosis assays were performed using FRTL-5 cells cultured on filters in dual chambered devices, with megalin expression only on the upper surface of the layers. TG was added to the upper chamber and transcytosed TG was measured in fluids collected from the lower chamber after incubation at 37 C. Treatment of FRTL-5 cells with CQ or AC did not affect binding and uptake of TG, but it did reduce T3 release from exogenously added TG, used as a measure of TG degradation in the lysosomal pathway. Treatment with CQ or AC resulted in an increase of transcytosis of TG across FRTL-5 cells, but only to a minimal extent (15-20%). The effects of CQ or AC and those of a megalin competitor (the monoclonal antibody 1H2, which reduced transcytosis) were not additive, suggesting that CQ and AC act on the megalin-mediated pathway. In conclusion, because TG binding to megalin is greatest at acidic pH, it is possible that TG does not dissociate from megalin in the lysosomal pathway. However, the pH-dependence of TG binding to megalin does not account for much of transcytosis, which probably occurs largely because of other mechanisms of targeting.


Heterogeneity of the thyroglobulin epitopes associated with circulating thyroid hormone autoantibodies in hashimoto’s thyroiditis and non-autoimmune thyroid diseases.

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We previously implicated TG leakage from fine-needle aspiration biopsy (FNAB) as responsible for circulating thyroid hormone autoantibodies (THAb). However, THAb were not always associated with TGAb. In the literature these negative findings have been interpreted against a role of TG as the antigen for THAb. To evaluate the TGAb status more fully and to gain information on TG epitopes involved in THAb development, we measured: 1) TGAb with an independent hemagglutination assay (HA), and 2) epitope specificity in a competitive ELISA, using 2 monoclonal Abs (mAb) against TG: mAb 42C3 and mAb 134C2. mAb 42C3 recognizes a cross-reactive iodinated epitope, whereas 134C2 is specific for human TG. We tested 12 Hashimoto’s thyroiditis (HT) and 35 non-HT patients sampled prior to, 1 and 3 months after FNAB. We found that, irrespective of thyroid disease or post-FNAB THAb status, certain patients previously classified as TGAb negative by IRMA tested TGAb positive by HA or by competition ELISA and vice versa. A post FNAB positive response to the 42C3 iodinated epitope in only one THAb IgM-T4+ve HT and a few THAb negative non-HT patients was observed. Furthermore, we observed that the 3 non-HT patients who expressed IgM-T3 THAb failed to bind either TG-mAb epitope. We conclude that a single TGAb assay is not sufficient to define the TGAb status, which can be achieved reliably only by using multiple TGAb assays. In addition, the TG-iodinated epitope recognized by 42C3 is not a major epitope in post-FNAB THAb, and the T3-epitope involved in THAb remains distinct from the mAb epitopes. In light of recent data in the literature, we further suggest that the responsible epitopes are more likely to be expressed in leaked TG fragments, rather than leaked intact TG.


Individual recombinant thyroglobulin type-1 domains are substrates for lysosomal cysteine proteinases.

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Thyroglobulin contains 11 repeats of a motif called thyroglobulin type-1 domain that show sequence similarity to some proteins exhibiting inhibitory activity against cysteine proteases. Here we report that thyroglobulin decreases the activity of cathepsins B, H, L, and papain. To examine the possible involvement of particular type-1 domains in that decrease of activity, some individual thyroglobulin type-1 domains were expressed in E. coli. These recombinant domains proved to be substrates for cathepsins B, H, L, and papain instead of inhibitors. The cleavage points with cathepsins B and L on the second and the fourth domains were determined. The possible reasons for degradation are discussed.
Molecular Biology & Immunology


**Delineation of five thyroglobulin T cell epitopes with pathogenic potential in experimental autoimmune thyroiditis.**

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Experimental autoimmune thyroiditis (EAT) is a T cell-mediated disease that can be induced in mice after challenge with thyroglobulin (Tg) or Tg peptides. To date, five pathogenic Tg peptides have been identified, four of which are clustered toward the C-terminal end. Because susceptibility to EAT is under control of H-2A(k) genes, we have used an algorithm-based approach to identify A(k)-binding peptides with pathogenic potential within mouse Tg. Eight candidate synthetic peptides, varying in size from 9 to 15 aa, were tested and five of those (p306, p1579, p1826, p2102, and p2596) were found to induce EAT in CBA/J (H-2k) mice either after direct challenge with peptide in adjuvant or by adoptive transfer of peptide-sensitized lymph node cells (LNCs) into naive hosts. These pathogenic peptides were immunogenic at the T cell level, eliciting specific LNC proliferative responses and IL-2 and/or IFN-gamma secretion in recall assays in vitro, but contained nondominant epitopes. All immunogenic peptides were confirmed as A(k) binders because peptide-specific LNC proliferation was blocked by an A(k)-specific mAb, but not by a control mAb. Peptide-specific serum IgG was induced only by p2102 and p2596, but these Abs did not bind to intact mouse Tg. This study reaffirms the predictive value of A(k)-binding motifs in epitope mapping and doubles the number of known pathogenic T cell determinants in Tg that are now found scattered throughout the length of this large autoantigen. This knowledge may contribute toward our understanding of the pathogenesis of autoimmune thyroiditis.


**A tandemly repeated thyroglobulin core promoter has potential to enhance efficacy for tissue-specific gene therapy for thyroid carcinomas.**


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Recombinant adenoviruses, carrying herpes simplex virus thymidine kinase (HSVtk) genes, were developed to evaluate the possibility of tissue-specific gene therapy for thyroid carcinomas. The HSVtk gene was driven by a minimal thyroglobulin (TG) promoter (AdTGtk) and a tandemly repeated minimal TG promoter (Ad2 x TGtk) to obtain thyroid-specific cell killing ability. The transduction of HSVtk genes by infection with Ad2 x TGtk followed by ganciclovir (GCV) treatment showed more powerful cytotoxicity for TG-producing FRTL5 cells, a rat normal thyroid cell line, and FTC-133 cells, a human follicular thyroid carcinoma cell line, than when infected with AdTGtk in vitro. The cell killing ability of Ad2 x TGtk was 10- to 30-fold higher than that of AdTGtk and similar to that of AdCMVtk, which carries HSVtk under the control of CMV promoter. Whereas after treatment with adenovirus/GCV to non-TG-producing cell lines (undifferentiated thyroid carcinoma cell lines and carcinoma cell lines from other tissues), Ad2 x TGtk and AdTGtk needed more than 100-fold concentrated GCV to reach IC(50) compared to AdCMVtk. We confirmed the enhanced efficacy of Ad2 x TGtk for tissue-specific cytotoxicity in vivo. After adenovirus/GCV treatment for FTC-133 tumor-bearing nude mice, Ad2 x TGtk enhanced tumor growth inhibition and survival rates compared to AdTGtk. Tumor growth inhibition and survival rates by Ad2 x TGtk were similar to that by AdCMVtk. Moreover, any toxic effect for rat normal tissues was not revealed after intravenous injections with Ad2 x TGtk and intraperitoneal administrations with GCV in vivo, whereas severe liver damages were observed after treatment with AdCMVtk/GCV. These data indicate a beneficial effect of Ad2 x TGtk for tissue-specific gene therapy for TG-producing thyroid carcinomas without toxicity for normal tissues.

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**Genotyping and characterization of two polymorphic microsatellite markers located within introns 29 and 30 of the human thyroglobulin gene.**

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The purpose of the present work was to characterize two new polymorphic microsatellite markers in the thyroglobulin gene. TGrI29 and TGrI30 repeats are located within introns 29 and 30, respectively. Genetic studies were carried out by using polymerase chain reaction (PCR) followed by denaturing polyacrylamide gel electrophoresis. TGrI29 exhibited clearly 4 distinguishable alleles ranging from 197 to 203 base pair (bp) in length and TGrI30 showed 8 alleles ranging from 502 to 542 bp. We characterized the two markers by determining allele frequencies and measures of variation. The heterozygosities (HET) observed of TGrI29 and TGrI30 were 0.522 and 0.522, respectively. The polymorphism information contents (PIC) were 0.471 and 0.434, respectively. No significant differences from Hardy-Weinberg values were found for these two systems. The PCR products of each allele were cloned using the pGEM Easy vector and directly sequenced by Taq polymerase-based chain terminator method. Sequencing analysis indicated that both loci are complex repeats, TGrI29 containing two types of variable motifs (tc)n and (tg)n, and TGrI30 a tetra-nucleotide tandem units (atcc)n. In two TGrI29 alleles and one TGrI30 allele were found two different subtypes in each one, with the same molecular weights but different distribution of the tandem repeats. In conclusion, both microsatellites analyzed are highly informative polymorphic markers and can be used in linkage studies in families with congenital hypothyroidism or autoimmune thyroid diseases.
Molecular Biology & Immunology

Role of protein disulfide isomerase in molecular fate of thyroglobulin and its regulation by endogenous oxidants and reductants.

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The molecular fate of thyroglobulin (Tg) is controlled by oligomerization, a means of storing Tg at high concentrations, and deoligomerization. The oligomerization of bovine Tg are intermolecular reactions that occur through oxidative processes, such as disulfide and dityrosine formation, as well as isopeptide formation; disulfide formation is primarily responsible for Tg oligomerization. Here, the protein disulfide isomerase (PDI) and/or peroxidase-induced oligomerization of unfolded thyroglobulins, which were prepared by treating bovine Tg with heat, urea or thiol/urea, was investigated using SDS-PAGE analyses. In addition, the enzymatic oligomerization was compared with non-enzymatic oligomerization. The thermally-induced oligomerization of Tg, dependent on glutathione redox state, was affected by the ionic strength or the presence of a surfactant. Meanwhile, PDI-catalyzed oligomerization, time and pH-dependent, was the most remarkable with unfolded/reduced Tg, which was prepared from a treatment with urea/DTT, while the thermally-unfolded Tg was less sensitive. Similarly, the oligomerization of unfolded/reduced Tg was also mediated by peroxidase. However, PDI showed no remarkable effect on the peroxidase-mediated oligomerization of either the unfolded or unfolded/reduced Tg. Additionally, the reductive deoligomerization of oligomeric Tg was exerted by PDI in an excessively reducing state. Based on these results, it is proposed that PDI catalyzes the oligomerization of Tg through the disulfide linkage and its deoligomerization in the molecular fate, and this process may require a specific molecular form of Tg, optimally unfolded/reduced, in a proper redox state.

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Isolation of a normal human thyroid cell line: hormonal requirement for thyroglobulin regulation.

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The long-term culture of functional follicular cells from normal adult human thyroid tissue has been obtained. They were expanded using a 1:2 split ratio until passage 28 (present status) in Click-RPMI medium enhanced with 5% fetal calf serum and diverse associations of hormones or components including porcine insulin and bovine thyrotropin. At passages 10 and 20, chromosome countings showed a normal diploid number and a normal karyotype. In calf serum containing media, cells are epithelial in the presence of thyrotropin (TSH) but present a slight elongated form in the absence of TSH. In serum-free media, 30 minutes after TSH stimulation, both epithelial and elongated cells changed in morphology to stellate-shaped, arborized forms, indicating the presence of functional TSH-receptors even in long term (18 months) TSH-free cultures. Cells produce thyroglobulin constitutively and large amounts of thyroglobulin are easily recovered in TSH-supplemented media, especially in the presence of insulin. Thyroglobulin production was increased versus days under TSH or insulin stimulation. Combination of the two hormones clearly resulted in a synergistic and not an additive effect. The other hormones present in the 6H components (transferrin, glycyllhistidyl-lysine, somatostatin, and hydrocortisone) had no positive effect on thyroglobulin accumulation in media in our experimental conditions. Addition of TSH to hormone-free cultures or to insulin-, insulin plus hydrocortisone-, or 5H-containing cultures resulted in a clear increase in thyroglobulin production. Withdrawal of TSH from 6H cultures resulted in a decrease in thyroglobulin accumulation in media. Six months were required to select fibroblast-free cultures and to get passage 6. But only 17 months separated passage 6 to passage 28, indicating that the proliferative rate is increasing with in vitro cell adaptation. Such normal adult thyroid cells, thyroglobulin-producing, TSH, and insulin-sensitive, represent a new normal human thyroid cell line allowing comparative studies with cells originating from pathologic thyroid tissues.
Detection of thyrotropin-receptor messenger ribonucleic Acid (mRNA) and thyroglobulin mRNA transcripts in peripheral blood of patients with thyroid disease: sensitive and specific markers for thyroid cancer.


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Because thyroid cancer cells express functional TSH receptors (TSHR), TSHR-mRNA in peripheral blood might serve as a tissue-/cancer-specific marker. We measured circulating TSHR-mRNA by RT-PCR in 51 normal controls, 27 patients with benign thyroid disease, 67 patients with treated differentiated thyroid cancer (DTC), and eight patients with newly diagnosed DTC, preoperatively. Results were compared with thyroglobulin (Tg) mRNA and serum Tg levels. TSHR-mRNA signals were not detected in normal controls and in 24 of 27 (89%) patients with benign thyroid disease. All 19 patients with treated DTC with evidence of distant or local disease tested positive for TSHR-mRNA (sensitivity 100%). Among patients with no evidence of disease, TSHR-mRNA was detected in 1 in 48 (specificity 98%). Six of the eight newly diagnosed DTC patients tested preoperatively were positive for TSHR-mRNA. The concordance between TSHR-mRNA and Tg-mRNA and between TSHR-mRNA and serum Tg was 95%. Fourteen patients with DTC (21%) had Tg antibodies, three with local disease (all positive for TSHR-mRNA), and 11 with no evidence of disease (all negative for TSHR-mRNA). Our results indicate that TSHR-mRNA and/or Tg-mRNA in peripheral blood are both equally sensitive and specific markers for monitoring thyroid cancer patients. Their principal value resides in the Tg antibody-positive patients in whom a positive or negative mRNA value might have indicated or obviated the need for a whole-body scan. Furthermore, the high specificity combined with their ability to predict thyroid cancer preoperatively suggests a potential role in detecting thyroid cancer in patients with thyroid nodules.

Diagnostics

Challenges of serum thyroglobulin (tg) measurement in the presence of tg autoantibodies.

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Real-time quantitative PCR measurement of thyroglobulin mRNA in peripheral blood of thyroid cancer patients and healthy subjects.

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Follow-up of recurrent differentiated thyroid carcinoma involves the measurement of serum thyroglobulin (Tg). However, Tg autoantibodies are present in a high proportion of thyroid carcinoma patients (up to 25%) and these can interfere with the Tg immunoassays. To overcome this obstacle, investigators have used real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) to measure Tg mRNA in the blood of patients with differentiated thyroid cancer, with varying degrees of success. In the present study, we demonstrate the first reported use of the PAXgene Blood RNA collection tube and extraction kit method for the preparation of RT-PCR-quality RNA with subsequent deployment of the latter in the development of a specific, sensitive, and reproducible Taqman assay for the detection and quantification of thyroglobulin mRNA. Beta-actin mRNA was also assayed and results are expressed as a ratio of Tg to beta-actin mRNA. The intra-assay coefficient of variations (CVs) for Tg and beta-actin mRNA assay were 27.7% and 25.4%, respectively. Inter-assay CVs were 20.8% and 28.8%, respectively, for the two assays. Tg mRNA was detected in all cancer subjects (n = 42) and healthy individuals (n = 20). Tg mRNA was significantly higher in cancer patients than in the healthy subjects (0.00169 +/- 0.00013 vs. 0.00051 +/- 0.00015; P = 0.0001). Fourteen cancer patients had detectable levels of serum Tg, and Tg mRNA levels tended to be higher in these than in cancer subjects with undetectable serum Tg (0.00188 +/- 0.00021 vs. 0.00157 +/- 0.000178; P = 0.08). Circulatory Tg mRNA measurement may serve a useful role in the assessment of thyroid cancer.


Low specificity of blood thyroglobulin messenger ribonucleic acid assay prevents its use in the follow-up of differentiated thyroid cancer patients.


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Thyroglobulin (Tg) is a glycoprotein specifically synthesized by follicular thyroid epithelium. After thyroideectomy and remnant (131)I ablation, serum Tg is a specific and sensitive marker for the presence of thyroid cancer tissue, and its measurement is fundamental in the follow-up of patients affected by differentiated thyroid carcinomas (DTCs), being even more sensitive than diagnostic whole-body scan. Unfortunately, serum Tg measurement becomes useless in approximately 15-25% of DTC cases who are positive for anti-Tg antibodies that interfere with the Tg measurement. In these cases, Tg mRNA measurement has been proposed as an alternative to serum Tg determination. The aim of this study was to verify the specificity and sensitivity of Tg mRNA measurement, performed by quantitative real-time RT-PCR, in a series of 100 subjects (80 DTC patients and 20 controls). From our data, the sensitivity and the specificity of the blood Tg mRNA measurement are 82.3 and 24.2%, respectively, with a positive predictive value and a negative predictive value of 65.6 and 43.7%, respectively. The comparison of the Tg mRNA with the serum Tg, measured by both chemiluminescent and ultrasensitive ELISA methods, confirmed the low specificity of the Tg mRNA assay. The hypothesis that Tg
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mRNA detectable levels could be predictive of future recurrences is not supported by the long follow-up (median, 7 yr; range, 3-29 yr) of our disease-free patients, who did not develop any recurrences in their clinical history. Moreover, nine disease-free patients, who showed positive levels of Tg mRNA (11.8-336 pg equivalents/ micro g RNA), were confirmed to be serum Tg free, both in basal conditions and after recombinant human TSH stimulation, 4 yr after the Tg mRNA detection. In conclusion, we demonstrated that the Tg mRNA assay is of poor utility in the follow-up of DTC patients. On the contrary, serum Tg measurement is a very sensitive and specific thyroid tumor marker, and we recommend that the follow-up of patients affected by DTC must be performed using serum Tg rather than blood Tg mRNA measurement.

Evaluation of quantitative measurement of thyroglobulin mRNA in the follow-up of differentiated thyroid cancer.

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Detection of thyroid cancer by thyroglobulin (Tg) assay in peripheral blood is useful in the absence of residual thyroid tissue, but it requires thyrotropin (TSH) stimulation for maximal sensitivity and is affected by circulating antithyroglobulin antibodies. To avoid these drawbacks, thyroglobulin mRNA (Tg mRNA) assay in circulating blood has been proposed. Initial studies showed that Tg mRNA assay was more positive in patients with metastasis than in cured patients. Further studies showed controversial data. We measured Tg mRNA in 26 patients undergoing levothyroxine (LT(4)) suppressive therapy after total thyroidectomy for thyroid cancer and in 11 controls. The stage of the cancer was defined according to the findings of the latest whole-body (131)I scan and serum Tg performed under LT(4) withdrawal. Patients were classified as cured (negative scan, negative stimulated Tg, 8 patients), with metastasis (positive scan in extrathyroid bed regions, positive Tg, 7 patients), with thyroid remnants (positive scan in thyroid bed, positive Tg, 8 patients), and discordant cases (negative scan, positive Tg, 3 patients). RNA was extracted from blood and analyzed by quantitative reverse transcription-polymerase chain reaction (RT-PCR) using two sets of primers and internal probes specific for Tg mRNA. This method allowed the detection of Tg mRNA in thyroid biopsies. Tg mRNA was undetectable in all control subjects and in all patients with cured cancer, positive in 1 of 8 patients with thyroid remnants, and in only 1 of 7 patients with metastasis. In conclusion, our data do not support the usefulness of Tg mRNA measurements in blood for monitoring thyroid cancer.

Serum thyroglobulin measurements with a high sensitivity enzyme-linked immuno sorbent assay: is there a clinical benefit in patients with differentiated thyroid carcinoma?

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Serial serum thyroglobulin (Tg) measurements with a highly sensitive enzyme-linked immuno sorbent assay (ELISA; functional sensitivity 0.03 ng/mL) in 126 patients (Tg autoantibody negative) with treated differentiated thyroid cancer (DTC) are described. At the beginning of the retrospective study, all 126 patients were in remission and Tg was detectable by ELISA in 92 (73%; range, 0.03-0.8 ng/mL). Over the following 4-year period, Tg levels remained essentially unchanged (i.e., any increases were less than 2 times the Tg level at the start of the study) in 121 of 126 (96%) and all 121 patients remained well. In 5 patients, Tg levels increased to more than 2 times the starting Tg level over the study period and in 4 of these 5, there was recurrence of DTC. The fifth patient in this group remains well as evidenced by extensive diagnostic imaging, although his serum Tg level continues to increase and can be stimulated by thyrotropin (TSH). Our results suggest that serial measurements of low levels of Tg by ELISA in treated patients with DTC enable detection of recurrence (without using TSH stimulation) 6-12 months earlier than would have been possible using a conventional Tg immuno-radiometric assay (IRMA). A prospective study is now needed to confirm these observations.

Critical aspects of immunoradiometric thyroglobulin assays.


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BACKGROUND AND AIM OF THE STUDY: Thyroglobulin (Tg) evaluation is currently used in the follow-up of patients with differentiated thyroid carcinoma (DTC), but the measurement methods are flawed by analytical inaccuracy. In this paper we describe the results of a comparison between seven different immunoradiometric assays (IRMAs) for Tg determination. MATERIAL AND METHODS: Tg was measured in 50 patients with DTC by means of the following commercially available IRMA kits: HTGK-2 (DiaSorin), Tg IRMA (Schering-CIS bio international), ELISA-hTg (Schering-CIS bio international), Tg IRMA C.T. (ICN Pharmaceuticals), SELeo Tg (Medipan Diagnostica), Tg Bridge IRMA (Adaltis) and IRMA-mat Tg (BYK-Sangtec Diagnostica). The distribution of the Tg values measured by the different IRMAs was compared and a correlation analysis was performed. RESULTS: The Tg values were widely dispersed and the classification of patients according to Tg concentrations of clinical relevance varied depending on the IRMA used. CONCLUSION: Despite efforts to develop standardized Tg assays, the measurement of this biomarker is still affected by a considerable degree of analytical inaccuracy. Tg values vary widely between assays and the classification of patients according to Tg values with clinical relevance is still dependent on the assay used.
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[Kon trollierliche Sensitivitätssteigerung in der Schilddrusenkarzinom-Nachsorge im Verlauf dreier Thyroglobulin-IMA-Generationen]


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Aim of our study was to evaluate the increasing sensitivity within three generations of thyroglobulin (Tg) assays, which were available during the past decade, and its clinical impact for patients with differentiated thyroid carcinoma. METHODS: Determination of Tg using the IRMA introduced in 1989 (DynoTest Tg, Henning Berlin, Berlin; assay A) and 1994 (Selco Tg, Medipan Diagnostica, Selchow; assay B), as well as the IEMA available recently (Medizym Tg Rem, Medipan Diagnostica, Selchow; assay C). RESULTS: We found a close correlation between the measurable Tg values of assay A and B (r=0.983; p<0.001) as well as assay B and C (r=0.976; p<0.001). Assay B (lowest detection limit: 0.3 ng/ml) was more than twice as sensitive as assay A and did not show quite as many disturbances of recovery (in 0.5% versus 4% of our patients). Due to its strict calibration to the European reference preparation CRM 457, Tg values determined by assay C were in the mean 1.9-fold higher than by assay B. Thus, with its functional sensitivity of 0.03 ng/ml assay C is nearly 20-fold more sensitive than assay B. Whereas the proportion of measurable Tg values was only 22% in a selected group of patients (criterion of inclusion: Tg in assay B < 1 ng/ml with TSH-suppressive conditions; n=317 serum samples from 103 patients), it was 68% in assay C, with good intra- and interindividual reproducibility of these values in the course. CONCLUSION: The ultrasensitive assay C is especially suitable for the follow-up of treated thyroid cancer patients being considered as cured, and may shorten the time interval until the detection of a recurrence markedly: the gain of time calculated from the Tg courses in patients with a gradually progressive tumor relapse ranged from 5 to 15 months.


Discordant serum thyroglobulin results generated by two classes of assay in patients with thyroid carcinoma: correlation with clinical outcome after 3 years of follow-up.

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BACKGROUND: Serum thyroglobulin measurement is an integral part of monitoring patients with thyroid carcinoma, but analytic problems pose serious difficulties in the utility of this test. METHODS: Between 1997 and 1998, serum samples from 83 patients with differentiated thyroid carcinoma were collected. Serum thyroglobulin was assayed by both radioimmunoassay and by an immunoradiometric assay. The disease status of patients with discordant serum thyroglobulin results was assessed in June 2001. Therefore, the predictive value of a single thyroglobulin measurement was assessed by evaluating the clinical status of patients 3 years later. RESULTS: Discordant serum thyroglobulin results were noted in 17 (20.4%) patients. Of the 17 patients with discordant results, 16 had adequate clinical follow-up data. Of these 16 patients, 11 patients had detectable levels of serum thyroglobulin by immunoradiometric assay (range, 1.4-350 microg/L) whereas levels were undetectable by radioimmunoassay (< 1 microg/L). All 11 patients had evidence of metastases 3 years later. Two patients had undetectable serum thyroglobulin levels using the immunoradiometric assay (< 1 microg/L), whereas they had detectable levels using radioimmunoassay (serum thyroglobulin 7.2-30 microg/L). The serum samples from both patients had normal recoveries and positive anti-thyroglobulin antibodies. Both patients developed metastases 3 years later. CONCLUSIONS: False-negative serum thyroglobulin results were significantly higher with the radioimmunoassay method compared with the immunoradiometric assay. The immunoradiometric assay is more reliable than the radioimmunoassay, particularly in patients who have no thyroglobulin antibodies. This finding is novel in that traditional immunoradiometric assay systems compared with radioimmunoassays usually have a higher incidence of false-negative results when assessed against clinical status. The immunoradiometric assay is subject to false-negative results in some patients with thyroglobulin antibodies, even when recovery experiments indicate the absence of interference. Thyroglobulin antibodies should be measured in all patients with differentiated thyroid carcinoma and if positive, results should be interpreted with extreme caution. Copyright 2003 American Cancer Society.DOI 10.1002/cncr.11472


Phantoms in the assay tube: heterophile antibody interferences in serum thyrogblobulin assays.

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Serum thyroglobulin (Tg) measurement is a major means of detecting thyroid cancer recurrence. Unlike anti-Tg autoantibody interferences, heterophile antibody (HAB) immunosassay interferences are not well recognized by laboratorians or clinicians as a Tg assay problem. When HAB interferences occur, they usually result in false negative test results. With the current trend to treat some thyroid cancer patients with radiodine on the basis of an elevated serum Tg result alone, this has the potential to result in unwarranted therapy. We evaluated the prevalence of HAB interference in a commonly used automated immunoassay in 1106 consecutive specimens with Tg values greater than 1 ng/ml. All Tg measurements were repeated after sample incubation in heterophile-blocking tubes (HBT). Results, which showed a more than 3 SD percentage difference from the original result, were considered to suffer from HAB interference. All possible interferences were confirmed by dilution testing. After HBT treatment, Tg levels dropped to less than 1 ng/ml in 32 specimens (P < 0.0000001), 20 of which fell to less than 0.1 ng/ml (P < 0.000002). Of these 20, 17 were anti-Tg autoantibody negative, and all 32 showed a fall of greater than 3 SD percentage (>56.9%) compared with the original result. There were also two samples that showed a significant increase of greater than 56.9% after HBT treatment. HAB interference is relatively prevalent (1.5-3%) in a commonly used automated Tg assay and can lead to clinically significant artifacts. It is currently unknown, but
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possible, that other immunometric Tg assays suffer from similar problems. Unless a Tg assay is confirmed to be free of HAB interference or uses additional blocking steps, as ours now does, HAB interference should be suspected if Tg results do not fit the clinical picture.


Thyroglobulin autoantibody levels below the cut-off for positivity can interfere with thyroglobulin measurement.

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Development of a dried whole-blood spot thyroglobulin assay and its evaluation as an indicator of thyroid status in goitrous children receiving iodized salt.

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BACKGROUND: Serum thyroglobulin appears to be a sensitive marker of thyroid dysfunction in endemic goiter. However, its value as an indicator of thyroid status in children after the introduction of iodized salt has not been tested. OBJECTIVE: The objective was to optimize and validate a thyroglobulin assay on dried whole blood spots and to evaluate thyroglobulin as an indicator of thyroid response to iodized salt. DESIGN: A standardized, commercially available, sandwich fluoroenzymometric serum thyroglobulin assay was adapted for use on blood spots and validated in Swiss children. In a 1-y prospective study in 377 goitrous Moroccan children aged 6-15 y, the assay was used to measure thyroglobulin before and after the introduction of iodized salt. Urinary iodine, thyroid volume, thyrotropin, and thyroxine were measured, and regression was done with thyroglobulin as the dependent variable. RESULTS: Correlation between the blood spot and serum assays was excellent (r = 0.98). The SD of the difference between the blood spot and serum assays was 3.8 micro g/L; the median CVs for the blood spot assay in controls and samples were 6.3% and 14.4%, respectively. Median thyroglobulin was 24.5 (range: 0-328.8) micro g/L at baseline and fell significantly after the introduction of iodized salt to 6.2 (0-83.1) and 4.4 (0-47.1) micro g/L at 5 and 12 mo, respectively (P < 0.0001). Regression of urinary iodine and thyroid volume on thyroglobulin was highly significant at baseline and at 5 mo (P < 0.001). CONCLUSION: Thyroglobulin, measured in dried whole blood spots, may be a valuable indicator of improving thyroid function in children after supplementation with iodized salt.


Measurement of thyroglobulin mRNA in peripheral blood as an adjunctive test for monitoring thyroid cancer.


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AIMS: Monitoring treated patients with thyroid cancer for recurrent or metastatic disease is currently based upon the serial measurement of circulating plasma thyroglobulin (Tg) concentrations. However, the clinical usefulness of Tg immunoadsays is limited by poor sensitivity and interference from anti-Tg antibodies. This study investigated whether the detection of Tg mRNA in peripheral blood, using reverse transcriptase polymerase chain reaction (RT-PCR), is of value in the biochemical surveillance of patients with thyroid cancer. METHODS: RNA was extracted from peripheral blood of five normal controls, six patients with abnormal thyroid function tests, and 28 patients who had undergone thyroidectomy for well differentiated thyroid cancer. From each, an 87 bp product from base pair 262 to 348 in the cDNA sequence of the thyroglobulin gene was amplified by RT-PCR. RESULTS: Tg mRNA was detected in normal individuals and patients with thyroid cancer. In the group of patients studied, identification of metastatic thyroid tissue by radioiodine scanning correlated better with Tg mRNA assay results than with serum Tg concentrations (accuracy 84%/ v 75%). No interference from circulating Tg antibodies was apparent. In patients studied prospectively over a 12 month period, there was a significant correlation between detectable Tg mRNA in peripheral blood and the presence or absence of metastatic disease, as demonstrated by radioiodine scanning. CONCLUSIONS: These results suggest that detection of Tg mRNA in blood is a more sensitive marker for metastatic thyroid disease than Tg immunoadsay, and appears to be unaffected by the presence of circulating anti-Tg antibodies.


Quantitative detection of peripheral thyroglobulin mRNA has limited clinical value in the follow-up of thyroid cancer patients.

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BACKGROUND: As recurrences after treatment for differentiated thyroid cancer can occur many years after thyroidectomy, periodic monitoring of serum thyroglobulin (Tg) levels is performed in these patients. However, autoantibodies that can interfere with Tg immunoadsays occur in the blood of approximately 25% of these patients. Several earlier reports suggest that measuring Tg mRNA by reverse-transcriptase polymerase chain reaction (RT-PCR) could be of value, especially in patients with Tg autoantibodies. METHODS: Using an earlier described, real-time quantitative Taqman RT-PCR assay, Tg mRNA concentrations were assessed in peripheral blood taken from 58 patients treated for thyroid cancer and from two healthy controls. RESULTS: In all tested samples Tg mRNA could be found. No correlation between serum Tg protein and Tg mRNA could be found. Tg mRNA concentrations did not differ between serum
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Tg-negative and Tg-positive patients. No differences in the number of patients with high or low Tg/beta-actin ratios were found between the groups of patients without, (131I) uptake on whole-body scan, or patients with thyroid bed uptake, uptake elsewhere in the neck, or distant metastases with or without regional uptake (P = 0.871). CONCLUSIONS: We were not able to confirm earlier positive reports on the clinical value of Tg mRNA measurement for the monitoring of patients treated for thyroid cancer.


[Clinical evaluation of a new thyroglobulin immunoradiometric assay in the follow-up of differentiated thyroid carcinoma]


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AIM: Formal and clinical comparison of a new 3 (rd) -generation-Tg-IRMA (3-G-IRMA; Dynotest Tg-plus) with a conventional Tg-IRMA (3-G-IRMA; SELco Tg-assy) for patients with differentiated thyroid carcinoma. In addition we evaluated, if thyroglobulin (Tg) levels above a specific threshold concentration indicate the need for further investigations for residual disease. PATIENTS, METHODS: Tg concentration of 105 sera of 93 consecutive patients with a differentiated thyroid cancer was determined with both assays and compared at different cut-off values (Dynotest Tg-plus: 0.2, 1, 2 ng/ml; SELco Tg-assy: 0.5, 1, 2 ng/ml) with the clinical results in respect to the corresponding TSH concentration. RESULTS: Tg concentration did not show any significant difference (SELco Tg-assy 0.5 mg/ml, Dynotest Tg-plus 0.2 mg/ml). The Tg-values of both assays correlated with 97%. However, correlation of recovery in both assays was small (40%). The sensitivities and specificities of both assays at different cut-offs and TSH values did not reveal significant differences. In patients with TSH concentration > 30 micro U/ml the functional assay sensitivity was superior to arbitrary cut-offs in the decision to start further evaluations. CONCLUSIONS: In our study neither formal nor clinical significant differences between both Tg-arrays were found. In a hypothyroid patient (TSH > 30 micro U/ml, Tg concentration exceeding the functional assay sensitivity) further investigations for residual disease are warranted. Higher thresholds are of limited value, due to an unacceptably high rate of false negative results.


High-sensitive 2nd generation thyroglobulin immunoradiometric assay. Clinical application in differentiated thyroid cancer management.

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BACKGROUND: Circulating human thyroglobulin (hTG) measurements have a pivotal role in the management of patients affected by differentiated thyroid cancer (DTC). The present study was undertaken by employing a new developed high-sensitive hTG immunoradiometric assay to evaluate its diagnostic performance in patients affected by radically cured and relapsing DTC and to set the most appropriate cut-off point for DTC management. METHODS: We retrospectively selected 172 patients without signs of recurrence after primary treatment and 45 patients with recurrences from DTC. Sera samples were collected during l-thyroxine (T4) suppressive therapy (onT4) and 4 weeks after T4 withdrawal (offT4) and hTG measured by a specific high-sensitive IRMA assay (DYNOtest Tg-plus, BRAHMS Diagnostica GmbH, Berlin, Germany). Sera showing the presence of AbhTG or hTG-recovery less than 80% were excluded from the study. ROC curve analysis was performed to select the best cut-off levels and diagnostic performance of the marker evaluated. RESULTS: By using onT4 cut-off level of 0.2 ng/ml and offT4 cut-off level of 0.5 ng/ml we obtained a sensitivity/specificity/accuracy profile of 0.91/0.98/0.96 and 0.98/0.97/0.97, respectively. We found onT4-hTG false negative results in 4 patient with local recurrence (n=2) or cervical lymph-node metastasis (n=2) while only 1 patient with local recurrence showed negative offT4-hTG. However, onT4 and offT4-hTG false-negative results were observed in 9 and 5 patients when 1.0 ng/mL cut-off level was employed. CONCLUSIONS: On the basis of our data, we conclude that DYNOtest Tg-plus assay is very effective and accurate in the evaluation of patients with DTC.


Thyroglobulin mRNA quantification in the peripheral blood is not a reliable marker for the follow-up of patients with differentiated thyroid cancer.

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BACKGROUND: The detection of serum thyroglobulin (Tg) by immunoassay is widely used to detect residual, recurring or metastatic thyroid carcinoma tissue in patients with differentiated thyroid cancer (DTC) after total thyroidectomy and radioiodine therapy. However, this method requires thyroid hormone withdrawal to increase sensitivity and is limited by the interference of anti-Tg antibodies. To solve these problems, the detection of Tg mRNA from circulating thyroid cells by reverse transcription (RT)-PCR has been suggested as an alternative method. However, different previous reports show discrepant conclusions as to the clinical usefulness of Tg mRNA quantification. METHODS: We compared three methods of blood collection and RNA extraction, and quantified Tg mRNA (by real time RT-PCR) in the peripheral blood of a) probands without thyroid disease (n=42), patients with b) thyroid autonomy (n=15), c) Graves’ disease (n=22), d) euthyroid goiter (n=6), and in DTC-patients after thyroidectomy and radioiodine therapy c) with (n=16) and f) without (n=37) metastasis. As the use of citrate blood in combination with a subsequent separation of mononuclear cells showed a significantly better RNA yield than the extraction of RNA from EDTA or citrate blood without the separation of mononuclear cells, this was the method used. Total RNA was reverse transcribed with random hexamer primers and Tg mRNA was amplified by real time RT-PCR using specific primers and hybridization probes. The Tg mRNA concentrations were normalized to beta-actin mRNA concentrations. RESULTS: Mean circulating Tg mRNA for each group detailed above, expressed as the ratio of Tg to beta-actin

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concentrations x 1000, were: a) 2.3 (range 0.03-70.89), b) 0.25 (range 0.02-0.55), c) 0.31 (range 0.05-1.36), d) 0.18 (range 0.08-0.35),
c) 0.57 (range 0.03-3.03) and f) 0.17 (range 0.02-0.60). Furthermore, we found no correlation between serum Tg and Tg mRNA.

CONCLUSIONS: In summary, our data do not show significant differences in Tg mRNA expression between the investigated groups. Therefore, the detection and quantification of Tg mRNA in peripheral blood is unlikely to be suitable for the follow-up of DTC.
**Role of serum thyroglobulin measurement in patients with thyroid nodules.**

Perros P, Weightman DR.

**Reference intervals for free thyroxine, total triiodothyronine, thyrotropin and thyroglobulin for Quebec newborns, children and teenagers.**

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**OBJECTIVE:** Paediatric reference values, although essential for interpreting patients' results, are scarce. Moreover, they are often population- and instrument-dependent. We have measured free thyroxine (Free T(4)), total triiodothyronine (Total T(3)), thyroglobulin (Tg) and thyrotropin (TSH) in samples obtained from groups of newborns, children and adolescents. SUBJECTS AND METHODS: Blood samples collected from healthy children and teenagers (100 girls and 100 boys) of age groups ranging between 9-10, 11-14 and 15-17 years and selected randomly from a cohort representative of the Quebec population, were used. Samples from infants of age ranging between 1 day and 2 years (n = 99) were obtained from a hospital-based population with benign conditions unlikely to affect thyroid function. Variables were measured on the Access 2 immunosystem. RESULTS: Free T(4), Tg and TSH levels declined significantly with age. However, Total T(3) level presented a nonlinear variation with age, being lower in the first month of life.

**Elevation in serum thyroglobulin during prolonged Antarctic residence: effect of thyroxine supplement in the polar 3,5,3'-triiodothyronine syndrome.**

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Extended Antarctic residence (AR) is associated with an increase in serum TSH, a decrease in free T(4), and an increase in T(3) production and clearance. It is not clear whether these adaptations reflect changes in clearance alone or whether intrinsic thyroidal synthetic activity also changes. Thyroglobulin secretion is an independent marker of intrinsic thyroid activity whose kinetics are independent of those of T(3) and T(4). In this study we examined changes in Tg levels in healthy subjects before and during AR and their responses to thyroid supplementation to help determine whether alterations in thyroid activity, and not just kinetics of clearance, underlie the changes seen with the polar T(3) syndrome. In cohort 1, we compared measurements of TSH and Tg in 12 subjects before deployment and monthly for 11 months during AR. In cohort 2, we compared the same measurements in 12 subjects monthly for 11 months of AR. Subjects were randomized to receive either placebo or levothyroxine in cohort 1 for 7 months and in cohort 2 for 11 months. Tg increased over baseline during the first 4 months of AR by 17.0 +/- 4.6% and after 7 more months by 31.7 +/- 4.3% over baseline in the placebo group of both cohorts (P < 0.0002). When L-T(4) was taken, Tg returned to a value not different from baseline (4.5 +/- 3.9%). The percent changes from baseline in serum TSH and Tg during AR were highly correlated (P < 0.00003) in the placebo group for both cohorts. The rise in Tg with TSH and the reduction in Tg with L-T(4) provide evidence of target tissue response to TSH and further confirm the TSH rise as physiologically significant. The results also suggest that the adaptive changes in thyroid hormone economy with AR reflect TSH-dependent changes in thyroid synthetic activity, which may help explain a portion of the increases in T(3) production found with AR.

**Role of thyroglobulin in the pathogenesis of Graves' ophthalmopathy: the hypothesis of Kriss revisited.**

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One of the hypothesis to explain the pathogenesis of Graves' ophthalmopathy (GO) was formulated by Joseph P. Kriss in the early 1970s. He postulated that the initiating event in the pathogenesis of GO is the deposition and accumulation of thyroglobulin (Tg) in orbital tissues, followed by an autoimmune reaction against Tg. In the last 30 yrs several studies have addressed this hypothesis, through various, different experimental approaches, raising results that are both in favor and against the possibility that Tg plays a role in the pathogenesis of GO. The finding that intact Tg is present in orbital tissues of GO patients supports Kriss' hypothesis, although the role of Tg as an autoantigen seems to be unlikely, as GO is not significantly associated with serum TgAb and mice immunized with Tg do not develop GO. Whether Tg is indeed involved in the pathogenesis of GO remains to be established. Our current view is that, provided that Tg plays a role, it is unlikely the only factor involved and Tg in orbital tissues may rather reinforce or worsen a damage initiated by other mechanisms.
Relations between various measures of iodine intake and thyroid volume, thyroid nodularity, and serum thyroglobulin.


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BACKGROUND: Iodine intake can be measured in various ways, and each method may have advantages and disadvantages. OBJECTIVE: We sought to investigate the potential associations of various measures of iodine intake with thyroid volume, prevalence of thyroid nodules, and serum thyroglobulin. We also sought to identify, if possible, groups at risk of thyroid disease because of their food choices. DESIGN: This cohort study included 4649 randomly selected subjects with mild-to-moderate iodine deficiency; the subjects lived in 2 cities in Denmark. Iodine intake was estimated by using a food-frequency questionnaire and by measuring iodine excretion in spot urine samples. Thyroid volume and nodularity were measured with ultrasonography. RESULTS: In multiple linear regression models, significant inverse relations were found between thyroid volume and estimated 24-h iodine excretion, iodine intake from diet plus supplements, iodine intake from diet/kg body wt, and milk intake (P = 0.001 for all), but not urinary iodine excretion measured as a concentration (P = 0.40). All measures of iodine intake were significantly related to serum thyroglobulin concentration (P <0.002), but only some measures of iodine intake were significantly related to the prevalence of thyroid nodules. CONCLUSIONS: Even in a geographic area where mild iodine deficiency is common, a significant relation between iodine intake and thyroid volume was found. All measures of iodine intake, except iodine excretion measured as a urinary concentration, predicted thyroid volume. Serum thyroglobulin concentration appears to be a good marker of iodine status. Subgroups with low intakes of milk and milk products had an increased risk of thyroid disease.