Autoantibodies to Thyroxin and Triiodothyronine in the Immunoglobulin G Fraction of Serum

Lorella Li Caizì, Salvatore Benveniga, Salvatore Battilato, Ferruccio Santini, and Francesco Trimarchi

Thyroid hormone antibodies (THAbs)—i.e., antibodies to thyroxin (T₄) and triiodothyronine (T₃)—are detected rarely in human serum, where they are searched for, possibly because of a quantitatively minimal interaction between thyroid hormones (the hapteners) and serum IgGs (the antibodies). The weak binding could result from these facts: (a) there are already six physiological carrier proteins for thyroid hormones; (b) THAbs usually account for a very small fraction of the total serum IgGs; (c) THAbs may have—as reported in the literature—a relatively low affinity. To ascertain whether THAbs could pass undetected in serum, we measured antibodies to T₃ and T₄ in both the serum and the corresponding IgG fraction of six normal persons and 45 patients with various thyroid diseases (Graves’ disease, idiopathic myxedema, Hashimoto’s thyroiditis, subacute thyroiditis, tumors), using radioimmunoprecipitation. The prevalence of antibodies to T₃ was 0/51 in both the sera and the IgG fractions; the prevalence of antibodies to T₄ was 1/51 in both materials. Because all of the sera that tested THAb negative were confirmed to be so in the THAB assay of the IgG fraction, we conclude that the prevalence of serum THAbs is not underestimated and that autoimmunization against thyroid hormones is really a rare phenomenon.

Additional Keyphrases: Graves’ disease · radioimmunoprecipitation · binding proteins

We reasoned that a simple way to ascertain whether the routine system of searching for THAbs in serum or plasma could underestimate their real prevalence in the circulation was to assay them in the isolated IgG fraction of serum, a material devoid of the above six carrier proteins but where THAbs, if present, would be concentrated. Our results, obtained by searching for THAbs with the radioimmunoprecipitation technique, show that none of the subjects who tested THAb negative in the serum assay gave a THAb-

positive result for the assay performed on the isolated IgG fraction. This indicates that autoimmunization against thyroid hormones is really a rare phenomenon.

Materials and Methods

We compared the prevalence of antibodies to T₃ and antibodies to T₄ in the original serum samples (stored at −20 °C) and in the corresponding IgG fraction from the following 51 subjects (76.5% of whom were females): six normal (euthyroid) persons, 21 with Hashimoto’s thyroiditis, nine with Graves’ disease, six with idiopathic myxedema, three with subacute thyroiditis, and six with thyroid tumors. The proportion of autoimmune thyroid diseases in the study group was large (36 of the 51) because we were aware that in these diseases THAbs are more frequent than in the other thyroid diseases (1).

None of the patients was being treated with T₃, T₄, or thyroid extract. Such treatments may saturate THAbs and mask their presence in the circulation (1). The overall prevalence of circulating thyroglobulin antibodies in the study group was 71.7%, as assessed with an immunoradiometric assay kit (Sorin, Saluggia, Italy).

IgGs were isolated by precipitation with ammonium sulfate and purified by affinity chromatography on diethylaminoethyl-Sephadex A-50 (5). We preferred this method to that involving Protein A, which does not allow isolation of the IgG3 subclass (4), a subclass that is frequently part of the THAbs (5). We had previously ascertained that IgGs so isolated are not contaminated by any of the above-mentioned carrier proteins. The mean concentration of the isolated IgGs was 13.2 (SD 5.3) g/L, with no statistically significant differences among the six groups studied.

THAbs in both the serum and its corresponding IgG fraction were detected with the well-validated (5, 6) radioimmunoprecipitation technique, which, unlike other, nonspecific, precipitation techniques (1), specifically immunoprecipitates the T₃- or T₄-IgG complex, because an antiserum to human IgG (Behringwerke, Marburg, F.R.G.) is used. 8-Anilinonaphthalene sulfonic acid was not added in the equilibration mixture (5), because it displaces the thyroid hormones from the major transport proteins in plasma to other proteins, including immunoglobulins, thus producing an artificial redistribution of the hormones and making unreliable any comparison between THAB assay performed on serum and THAB assay performed on the IgG fraction. Results are expressed as the percent bound (immunoprecipitated) of the total radioactivity, after subtraction of the radioactivity precipitated in the absence of the antisemum to IgGs. Antibodies to T₃ in both the serum and the IgG fraction were assayed in duplicate in the same run, as were antibodies to T₄ in a separate run. The same batch of antisemum to IgGs was used throughout this study.

Results and Discussion

Table 1 shows that the percentage of ¹²⁵I-T₄ immunoprecipitated both in serum and in the IgG fraction of the five disease groups did not differ statistically from the corresponding percentages of the normal group. The same was

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3 Nonstandard abbreviations: THAbs, thyroid hormone antibodies; T₃, thyroxin; T₄, triiodothyronine.
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5 Received August 1, 1988; accepted September 19, 1988.
true, except for the samples from Graves’ disease patients, for $^{[125]}T_4$. These low percentages reflect the known tendency of thyroid hormones to interact nonspecifically with proteins.] THAbs were undetectable in all the serum samples and the corresponding IgG fractions, except for one sample from a patient with Graves’ disease (see below). Therefore, the prevalence of the THAbs was the same in both sera and the corresponding IgG fractions (antibodies to $T_4 = 0.51$, antibodies to $T_3 = 1.51$). Thus, for a person who tests THAb negative with one THAb assay in serum, it is not necessary to repeat the assay in the isolated IgG fraction from that person.

For one patient with untreated Graves’ disease, antibodies to $T_2$ were detected in the serum and, as expected, in the corresponding IgG fraction: $^{[125]}T_2$ immunoprecipitated = 25.8% and 51.2%, respectively. Such high percentages account for the large standard deviation in the Graves’ disease group (Table 1). This patient had a clearly increased serum $T_2$ (296 nmol/L), but a falsely normal $T_3$ concentration (24 nmol/L). Both hormones were assayed with commercial RIA kits in which polyethylene glycol was used to separate bound from free hormone.

In conclusion, our study shows that the rarity with which THAbs are detected in human serum is real. In addition, because the search for THAbs in serum has the same sensitivity as for that in the IgG fraction, and because isolation of the latter necessitates additional time and cost, screening the IgG fraction for THAbs has no practical utility.

References

Carboxyhemoglobin as Measured by Gas Chromatography and with the IL 282 and 482 CO-Oximeters
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We measured the concentration of carboxyhemoglobin (HbCO) in original and CO-tonomerized blood samples from 53 intensive-care patients with IL 282 and IL 482 CO-Oximeters and by gas chromatography (GC), finding very strong correlations among the three methods for HbCO concentrations >2.5%. For concentrations within the normal reference interval ≤2.5%, however, the correlation between CO-Oximetry and GC is poor ($r^2<0.26$). The capillary mode of the IL 482 has a consistently lower correlation with any other method or mode. The correlations of measurements between the CO-Oximeters for total hemoglobin and oxyhemoglobin were excellent ($r^2=0.98$). Correlations for methemoglobin were lower, owing to its low concentrations in the samples. We conclude that the IL 482 and the IL 282 are analytically equivalent for all analytes measured, but that both instruments differentiate poorly between HbCO values that fall within the reference interval.

For years, routine clinical measurements for carboxyhemoglobin (HbCO) have been made with the IL 282 CO-Oximeter (Instrumentation Laboratory, Inc., Lexington, MA), a four-wavelength spectrophotometric instrument. The performance of this instrument has been correlated with that of the previous model, IL 182 (1), and of other instruments (2) and techniques (2–6), and more recently, with its successor, the IL 482 (7). We have developed a microsample method for determination of HbCO in blood by gas chromatography (8, 9), a technique not subject to the interferences reported for spectrophotometric methods. Although the correlation between HbCO measurements by CO-Oximeter and GC is excellent when a wide range of concentrations is considered, this correlation deteriorates for comparisons of HbCO values that fall within the reference interval (9). Furthermore, fetal hemoglobin (HbF) was

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Table 1. $^{[125]}T_4$ or $^{[125]}T_3$ Immunoprecipitated in Serum or in Its Corresponding IgG Fraction from Six Normal Persons and 45 Patients with Thyroid Diseases

<table>
<thead>
<tr>
<th></th>
<th>Hashimoto’s</th>
<th>Graves’</th>
<th>Subacute</th>
<th>Myxedema</th>
<th>Thyroiditis</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum $^{[125]}T_4$</td>
<td>3.2(0.8)</td>
<td>3.3(0.6)</td>
<td>3.2(0.4)</td>
<td>3.1(0.9)</td>
<td>3.3(0.3)</td>
<td>3.2(0.4)</td>
</tr>
<tr>
<td>Serum $^{[125]}T_3$</td>
<td>2.1(0.3)</td>
<td>2.1(0.4)</td>
<td>2.1(0.5)</td>
<td>1.9(0.1)</td>
<td>2.2(0.3)</td>
<td></td>
</tr>
</tbody>
</table>

Results are given as mean (SD) percent bound of the total radioactivity, after correction for nonspecific binding.

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