Serum levels of interleukin 6 and tumor necrosis factor-α in hyperthyroid patients before and after propylthiouracil treatment

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Contrary to the usual inhibitory role of tumor necrosis factor-α (TNF-α) in thyroid metabolism, it also has specific stimulatory effects in autoimmune thyroid disorders, including induction of HLA class II antigen-presenting cell--T cell interaction. Despite high intrathyroidal concentrations, various studies were not able to demonstrate high serum levels of TNF-α in patients with Graves' disease. To investigate this discrepancy we determined TNF-α and interleukin 6 (IL-6) levels in 25 hyperthyroid patients who responded to propylthiouracil treatment (16 with Graves' disease and nine with toxic multinodular goiter) and compared them with the levels found in euthyroid patients with simple diffuse goiter (n = 15) and normal healthy controls (n = 15). Median IL-6 levels were high in both Graves' disease and toxic multinodular goiter patients before propylthiouracil treatment (23 and 26.5 pg/ml, respectively). After restoring euthyroidism there was a statistically significant decline to near-normal levels (3 and 10 pg/ml, respectively). On the other hand, median serum TNF-α levels were high only in Graves' disease patients (20 pg/ml) and could not be normalized with antithyroid medication (20 pg/ml) compared to that of controls (5 pg/ml). Tumor necrosis factor-α, but not IL-6, was found to be high in the sera of Graves' disease patients when euthyroid, which may be due to an ongoing antigen--antibody interaction, a feature of autoimmune attack. It remains to be determined whether the degree of TNF-α and/or IL-6 elevation will be a predictor of disease recurrence.

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Cytokines are known to mediate processes that are important in the initiation and continuation of thyroid autoimmunity, including the induction of HLA class II expression on thyroid epithelial cells by interferon gamma and tumor necrosis factor-α (TNF-α) (1, 2). Autoantigen-reactive cells, upon stimulation by class II-expressing thyrocytes acting as antigen-presenting cells, release cytokines and maintain thyrocyte class II expression and antigen-presenting function (3).

Intrathyroidal cytokine production is not restricted to thyroid-infiltrating mononuclear cells; it may also involve thyroid follicular cells (4). In particular, thyroid cells have been found to produce interleukin 6 (IL-6) (5). This is a cytokine with key roles in B cell terminal differentiation and T cell activation (6). Although intrathyroidal IL-6 levels are increased in Graves' disease, high circulating IL-6 levels have not been detected in a small group of patients with Graves' hyperthyroidism, the authors arguing that increases in tissue levels may be insufficient to be reflected in sera (7).

Tumor necrosis factor-α receptors have been demonstrated on thyroid epithelial cells (8). Furthermore, TNF-α influences growth and function of these cells (9).

Moreover, the induction of HLA class II expression on thyrocytes by TNF-α suggests a mediator function in thyroid autoimmunity. However, conflicting results have been reported regarding intrathyroidal concentrations of TNF-α in Graves' disease (4, 10) and serum levels have been detected within the normal range (11).

The aim of this study was to investigate the serum levels of cytokines and to determine how levels would be affected by treatment with propylthiouracil (PTU).

Patients and methods

Subjects

In accordance with the Helsinki II Declaration, 40 consecutive patients and 15 healthy volunteers as controls gave informed consent to take part in the study after the study protocol was explained to them. None received any medications prior to and during the study or had any other illness.

Of the participating patients 16 had Graves' disease (six men and 10 women; mean age 39.3 ± 4.6 years), nine had toxic multinodular goiter (one man and eight women; mean age 40.4 ± 5.7 years) and 15 had non-
Table 1. Serum levels of thyroid hormones (T<sub>3</sub>, T<sub>4</sub>), cytokines (TNF-α, IL-6) and body mass indices (BMIs) according to groups before and after propylthiouracil treatment.<sup>a</sup>

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NTDG</th>
<th>TMNG</th>
<th>Graves&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; (nmol/l; mean±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.12 ± 0.38</td>
<td>2.28 ± 0.38</td>
<td>6.60 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.83 ± 2.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>After</td>
<td>--</td>
<td>--</td>
<td>2.32 ± 0.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.59 ± 1.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (nmol/l; mean ± SD)</td>
<td></td>
<td></td>
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<tr>
<td>Before</td>
<td>122.8 ± 26</td>
<td>101.6 ± 14</td>
<td>230.6 ± 62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>274.9 ± 46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>After</td>
<td>--</td>
<td>--</td>
<td>89.8 ± 30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>110.5 ± 39&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TNF-α (pg/ml; median [IQR])</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>5.0 [0.6]</td>
<td>5.4 [1.2]</td>
<td>5.0 [5.3]</td>
<td>20 [26]&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>After</td>
<td>--</td>
<td>--</td>
<td>5.0 [5.3]</td>
<td>20 [15]&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-6 (pg/ml; median [IQR])</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>6.0 [3.3]</td>
<td>5.7 [1.1]</td>
<td>26.5 [18]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.0 [30]&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>After</td>
<td>--</td>
<td>--</td>
<td>10.0 [17]&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.0 [13]&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;; mean ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>31.4 ± 4.1</td>
<td>30.7 ± 6.2</td>
<td>27.5 ± 4.5</td>
<td>28.5 ± 3.8</td>
</tr>
<tr>
<td>After</td>
<td>--</td>
<td>--</td>
<td>29.0 ± 4.2</td>
<td>30.1 ± 4.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> NTDG: non-toxic diffuse goiter; TMNG: toxic multinodular goiter; IQR: interquartile range.

<sup>b</sup> p < 0.0003 vs controls and NTDG group (Mann–Whitney U test).

<sup>c</sup> Decline after propylthiouracil treatment (p < 0.05: Wilcoxon matched-pairs test).

<sup>d</sup> p < 0.0003 vs TMNG group (Mann–Whitney U test).

toxic goiter (four men and 11 women, mean age 34.4 ± 4.7 years), all diagnosed according to previously published criteria (12). Healthy controls (nine men and six women) had a mean age of 34.3 ± 6.4 years. Body mass indices were calculated for hyperthyroid patients before and after treatment.

The mean duration of the patients’ symptoms was 1.1 ± 0.6 and 1.7 ± 0.5 years for Graves’ disease (GD) and toxic multinodular goiter (TMNG), respectively. No side effects or complications were observed during follow-up and all patients were able to complete the study.

**Blood sampling**

Baseline samples for thyroid hormones (T<sub>3</sub> and T<sub>4</sub>) TSH, TNF-α and IL-6 were drawn from the patients with GD and TMNG at the time of diagnosis and after 6 weeks of treatment with PTU (300 mg/day). All patients became euthyroid at the end of 6 weeks. Respective samples were drawn only once from non-toxic diffuse goiter patients and controls.

**Assays**

Blood samples were centrifuged immediately and the sera were stored at −53°C. Samples were assayed by the ELISA method using commercial kits (for TNF-α: human TNF-α ELISA kit, Janssen Biochimica, Belgium; for IL-6: enzyme immunoassay kit, Immunotech International, France). All cytokine assays were done in duplicate. Intra- and interassay coefficients of variation were 4.9% and 7.3%, respectively, for TNF-α and 4.4% and 5.6%, respectively, for IL-6. The threshold of detection for TNF-α was 5 pg/ml and that for IL-6 was 3 pg/ml.

**Statistics**

Assessment of data with undetectable or extreme values in cytokine assays led us to use the non-parametric Kruskal–Wallis test for the analysis of variances. Post hoc comparisons were performed by the Mann–Whitney U test with downward-adjusted p values (significant p values were assigned to 0.05 divided by 6, which in turn is 0.0083) (13). Significances of parameters within each group were tested by the Wilcoxon matched-pairs signed rank test (13). Correlations between variables within groups were tested by Spearman’s correlation test and p < 0.05 was accepted as significant. The statistical package for social sciences (SPSS) for Windows v5.01 was utilized to analyze the data. Values are expressed as median and interquartile range (IQR).

**Results**

Although hyperthyroid patients gained some weight when they became euthyroid, this increase did not reach statistical significance (Table 1).

Serum concentrations of thyroid hormones (T<sub>3</sub> and T<sub>4</sub>) and cytokines (TNF-α and IL-6) and body mass indices (BMIs) before and after PTU treatment are shown in Table 1. Thyroid hormone levels of GD patients were not significantly different from the levels of TMNG patients, both before and after treatment. After PTU therapy, T<sub>3</sub> and T<sub>4</sub> levels showed a significant decline (p < 0.0001) and were not different (p > 0.05) from the levels found in the euthyroid group, i.e. non-toxic diffuse goiter patients and controls.

In the group with TMNG, median TNF-α levels both before and after treatment were not statistically
Fig. 1. Interquartile ranges with median values (|) of serum TNF-α and IL-6 levels of controls (□), patients with non-toxic diffuse goiter (NTDG), (■) and levels before (⊙) and after (□) treatment in patients with Graves' disease and toxic multinodular goiter (TMNG): *p < 0.0083 vs controls and NTDG patients; †p < 0.05 vs levels before treatment; ‡p < 0.0083 vs controls, NTDG and TMNG patients.

different from controls. In this group, median pretreatment IL-6 levels were significantly high (p < 0.001) compared with post-treatment levels and the levels found in controls, whereas the last two values were not statistically different (Fig. 1).

In GD patients median levels of TNF-α before and after treatment were statistically higher (p < 0.001) than controls and non-toxic diffuse goiter patients. In this group, TNF-α levels before and after treatment showed a steady-state pattern despite antithyroid treatment. Similar to TMNG patients, IL-6 levels of these patients were significantly high before treatment and declined to a level comparable to controls following treatment (Fig. 1).

There were no significant differences in the cytokine levels by means of age between hyperthyroid patients or euthyroid controls. No significant difference was observed between non-toxic diffuse goiter patients and controls regarding the thyroid hormone or cytokine levels (p > 0.05).

Discussion

In this study we documented elevated serum cytokine concentrations in patients with hyperthyroidism due to Graves' disease and toxic multinodular goiter. Levels of IL-6 showed a similar pattern during treatment with PTU in both forms of hyperthyroidism. In accordance with its role in the inflammatory process, it has been shown that IL-6 is high during the acute phase of
hypothyroidism. The exact reason for the decrease in IL-6 is unclear, however, and one cannot exclude an effect of treatment (14).

The TNF-α levels showed a different pattern to IL-6. Unlike patients with Graves' disease, TNF-α levels were not high in patients with TMNG, which is a non-autoimmune thyroid disease. In hyperthyroidism due to Graves' disease, TNF-α levels were elevated and, although some decline was noted, they could not be reversed to normal after 6 weeks of antithyroid medication.

The role of TNF-α as a key mediator of cachexia has been the subject of intense debate. While TNF-α administration can cause weight loss in experimental animals, there is little evidence that human cachexia is specifically associated with changes in TNF-α levels (15). Our results also do not support such a hypothesis because we did not document significant weight changes in our patient groups and their BMIs were not different from those of controls.

High levels of TNF-α in the sera of the patients with Graves' disease may be due to a systemic reaction to disease and/or to an intrathyroidal antigen–antibody interaction, which is a feature of autoimmune diseases. Elevated levels of cytokines in the thyroid gland may induce autoreactive T cells to produce other cytokines, thus perpetuating the autoimmune response. This may lead to a spillover of TNF-α into serum.

In vitro, a reduced TNF response after repeated stimulation has been demonstrated (8). Paschke et al. could not detect any TNF-α in thyroid tissues from longstanding, relapsing Graves' disease patients, and suggested that TNF-α may be distinctly lower during the chronic phase of the disease (10). Similarly, several studies were not able to detect increased cytokine concentrations in the circulation and argued that tissue levels were not reflected in serum concentrations (4, 11). The discrepancy between previous studies and our study may be due to the use of a different methodology and a smaller patient group (4, 7, 11) and investigation during different phases of the illnesses. Studying TNF-α levels after a longer period of euthyroidism may be interesting and may show lower TNF-α levels.

Thyrostatic drugs may exert an inhibitory effect on thyroid epithelial cells, resulting in reduced expression and immunological signaling of surface major histocompatibility antigens. This drug-induced down-regulation of thyrocyte histocompatibility antigens may in turn reduce the rate of activation of T helper-like cells and transiently increase T suppressor-like cells (16, 17). The altered T cell activation profile and restoration of euthyroidism may be associated with reduced IL-6 levels but, as shown in this study, it does not seem to affect TNF-α levels.

In conclusion, serum levels of IL-6 are high in hyperthyroid patients and decline as disease activity subsides with an antithyroid therapy. Whether the thyroid status might affect the clearance or metabolic rate of this cytokine remains to be elucidated. The TNF-α levels are elevated only in patients with autoimmune thyroid disease, i.e. Graves' disease, and do not revert to normal after short-term PTU treatment.

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Authors' note. Serum levels of TNF-α and IL-6 are given in pg/ml. Because the molecular weight of IL-6 is not stable within solutions, owing to glycosylation and deglycosylation, expression of serum levels in molar concentrations was not possible.

References

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