COMMENT

High Levels of Circulating CXC Chemokine Ligand 10 Are Associated with Chronic Autoimmune Thyroiditis and Hypothyroidism

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CXC chemokine ligand 10 (CXCL10), an interferon-γ-inducible chemokine associated with Th1-mediated immune responses, has been proposed as a marker of inflammation in autoimmune diseases. We measured serum CXCL10 concentrations in 223 consecutive patients with newly diagnosed autoimmune thyroiditis (AT), 97 euthyroid controls, and 29 patients with nontoxic multinodular goiter and related this parameter to the clinical phenotype. The three groups were similar in gender distribution and age; among the AT patients, 24% had subclinical hypothyroidism. Serum CXCL10 level was significantly higher in AT patients (157 ± 139 pg/ml) than in controls (79 ± 38) or patients with multinodular goiter (90 ± 32; P < 0.0001). Among patients with AT, CXCL10 levels were significantly higher in those with a hypoechoic ultrasonographic pattern and hypothyroidism. In a multiple linear regression model including age, thyroid volume, hypoechogenicity, hypervascularity, TSH, free T₄, and antithyroid peroxidase, only age (standardized coefficient = 0.39; P = 0.0001) and TSH (standardized coefficient = 0.41; P < 0.0002) were significantly related to serum CXCL10 levels. We conclude that circulating CXCL10 is increased in patients with AT and is associated with hypothyroidism. CXCL10 may be regarded as a marker of a more aggressive thyroiditis leading to thyroid destruction. (J Clin Endocrinol Metab 89: 5496–5499, 2004)

CHEMOKINES ARE A group of peptides of low molecular weight that induce the chemotaxis of different leukocyte subtypes (1). The major function of chemokines is the recruitment of leukocytes to inflammation sites, but they also play a role in tumoral growth, angiogenesis, and organ sclerosis (2, 3). Chemokine effects are mediated by specific membrane receptors, and in general, one receptor binds more than one chemokine, and one chemokine binds to more than one receptor (4, 5). At present, more than 50 chemokines have been described, which have been classified into four major families (1). To date, only two of these families have been extensively studied and characterized, namely, the CC and CXC chemokines. The CXC chemokines inducible by interferon-γ [CXCL9 (CXCL10, and CXCL11] are associated with Th1-mediated immune responses and interact exclusively with the two alternatively spliced variants of the CXCR3 receptor, named CXCR3-A and CXCR3-B (6).

Interest in interferon-γ-inducible chemokines has arisen from studies of autoimmune thyroid disorders. In patients with Graves’ disease, the CXCR3 receptor was found to be highly expressed in endothelial cells as well as in infiltrating inflammatory cells, whereas CXCL10 was detected not only on these cells, but also on thyrocytes (7, 8). At the same time, it was shown that when stimulated by interferon-γ, human thyrocytes in primary culture as well as human mesangial and endothelial cells produce large amounts of CXCL10 (9, 10). In addition, by immunohistochemistry an increase in CXCL10 and CXCL9 was found in thyroid tissue specimens obtained from subjects affected by Hashimoto’s thyroiditis (9). Furthermore, increased CXCL10 mRNA expression was found in patients with recent onset of the disease, whereas in patients with long-standing disease, CXCL10 expression was comparable to that in controls (8). Serum CXCL10 concentrations are increased in several endocrine autoimmune conditions, including type 1 diabetes mellitus and Graves’ disease (8, 11–13). To our knowledge, no study has evaluated the interferon-γ-inducible chemokine status in patients with autoimmune thyroiditis (AT). The aim of the present study therefore was to measure serum CXCL10 levels in patients with chronic AT and relate the findings to the clinical phenotype.

Patients and Methods

Patients

From the outpatient clinic, we selected 223 consecutive Caucasian patients with newly diagnosed chronic AT (Table 1). The patients were...
referred to us by general practitioners or other hospitals because of the presence of circulating thyroid autoantibodies or hypothyroidism, or clinical suspicion of a thyroid disorder. The diagnosis of AT (14, 15) was established from the clinical presentation (presence of a firm goiter, varying in size from small to very large, with a lobulated surface), thyroid hormone and thyroid autoantibody measurements, and/or thyroid ultrasonography (decreased, dyshomogeneous echogenicity). The majority of these patients had a normal thyroid volume; some showed goiter (19%) or atrophic thyroiditis (10%). A minority of patients (7%) were submitted to fine needle aspiration (FNA) to exclude the presence of thyroid cancer or lymphoma; in these cases, cytology confirmed the presence of a lymphocytic infiltration.

Controls

Two controls groups were used. The first control group (controls I; n = 97) consisted of a random sample of the general population from the same geographic area (16) in whom a complete thyroid work-up (history, physical examination, TSH, free T4 (FT4), free T3 (FT3), anti-thyroglobulin (AbTg) and antithyroid peroxidase (AbTPO) antibodies, and ultrasonography) was available and excluded the presence of thyroid disorders. A second control group comprised 29 patients with nontoxic multinodular goiter extracted from the same random sample of the general population. The majority of these patients had a normal thyroid volume; some showed goiter (30%). All of these patients were submitted to FNA to exclude the presence of thyroid cancer or lymphoma; cytology confirmed the absence of a malignancy. In all patients and controls, a blood sample was collected in the morning, after overnight fasting, and serum was kept frozen until measurements of thyroid hormone, thyroid autoantibodies, and CXCL10.

All study subjects gave their informed consent to the study, which was approved by the local ethical committee.

Ultrasonography of the neck and FNA

Neck ultrasonography was performed by the same operator, who was unaware of the results of thyroid hormones, autoantibodies, and CXCL10 measurements, using a probe (Esaote, Florence, Italy; AU5 with a sectorial 7.5-MHz transducer). Thyroid volume was calculated using the ellipsoid formula, as previously described (14, 17, 18). The presence of hypoechoic and dyshomogeneous echogenicity was arbitrarily rated at three levels (0 = normal echogenicity; 1 = slight hypoechoic and dyshomogeneous; 2 = severely hypoechoic and dyshomogeneous) to evaluate structural abnormalities of thyroid tissue associated with thyroid autonomy (19, 20). The presence of thyroid nodules was recorded, and nodules with a diameter greater than 10 mm were submitted to ultrasonography-guided FNA, which was performed by the same operator, using a free-hand method as previously described (17, 20).

Thyroid blood flow (TBF)

TBF by color-flow Doppler (CFD) was studied in all patients (21). The CFD pattern was defined as: normal (or type 0), TBF limited to peripher-

### Table 1. Thyroid status of control subjects and AT

<table>
<thead>
<tr>
<th>No.</th>
<th>Control</th>
<th>Thyroiditis</th>
<th>Multinodular goiter</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>97</td>
<td>223</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43 ± 17</td>
<td>46 ± 15</td>
<td>45 ± 5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>24/76</td>
<td>14/86</td>
<td>22/78</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>11 ± 8</td>
<td>15 ± 13</td>
<td>18 ± 9</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>68</td>
<td>0</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>1.8 ± 1.0</td>
<td>3.1 ± 4.1</td>
<td>1.1 ± 0.7</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>8 ± 4</td>
<td>678 ± 1130</td>
<td>15 ± 8</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>9 ± 9</td>
<td>279 ± 400b</td>
<td>16 ± 19</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>81</td>
<td>0</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>76</td>
<td>0</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>24</td>
<td>0</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>79 ± 38</td>
<td>157 ± 139b</td>
<td>90 ± 32</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Serum TSH, µU/ml = mU/liter. NS, Not significant.

a P < 0.02 vs. controls.
b P < 0.05 or less vs. controls and vs. multinodular goiter.

Results

The demographic and clinical features of patients and controls are reported in Table 1. The mean CXCL10 level was significantly higher in patients with thyroiditis, particularly in hypothyroid patients, than in controls or multinodular goiter patients (Table 1), although some overlap was evident in the low range of CXCL10 values (Fig. 1). In AT patients, serum CXCL10 levels were significantly higher in patients older than 50 yr (196 ± 143 vs. 133 ± 131 pg/ml; P = 0.001), in patients with a hypoechoic pattern (176 ± 144 vs. 136 ± 86 pg/ml; P = 0.008), and in those with hypothyroidism (243 ± 131 pg/ml; type I, TBF mildly increased; type II, TBF clearly increased or type III, TBF markedly increased (21). In AT patients, TBF bore no relation to the thyroid status and was type 0 in 63%, type I in 31%, type II in 6% of patients; none of the patients had type III CFD pattern.

Laboratory evaluation

Thyroid function and thyroid autoantibodies were measured as previously described (14, 22). Circulating FT4 and FT3 were measured by commercial RIA kits (AMERLEX-MAB FT4, FT3, Kit, Amersham Biosciences, Little Chalfont, UK). Serum TSH (DiaSorin, Saluggia, Italy), AbTPO and AbTg (Valent Pharmaceuticals, Costa Mesa, CA) were evaluated by immunoradiometric assay methods. For AbTg, AbTPO, positivity was set at more than 50 and more than 10 UI/ml, respectively.

CXCL10 ELISA

Serum CXCL10 levels were assayed by a quantitative sandwich immunoassay using a commercially available kit (R&D Systems, Inc., Minneapolis, MN), with a sensitivity ranging from 0.41–4.46 pg/ml and a mean minimum detectable dose of 1.67 pg/ml. The intra- and interassay coefficients of variation were 3.0% and 6.9%, respectively.

Data analysis

Values are given as the mean ± sd for normally distributed variables, otherwise as the median and interquartile range (in parentheses). Mean group values were compared using one-way ANOVA for normally distributed variables (age and body mass index), otherwise by the Mann-Whitney U or Kruskal-Wallis test. Proportions were compared by the χ² test. Post hoc comparisons on normally distributed variables were made using the Bonferroni-Dunn test. Univariate and multivariate analyses were performed by multiple linear regression analysis using CXCL10 as the dependent variable and age, thyroid volume, TSH, FT4, AbTPO, hypoechoic pattern, and the presence of hypervascularity as covariates.
239 vs. 146 ± 103 pg/ml; \( P = 0.001 \), whereas no significant difference was observed in relation to the presence of atrophic thyroiditis, goiter, AbTPO positivity, AbTg positivity, or the presence of hypervascularity. In a multiple linear regression model including age, thyroid volume, TSH, FT\(_4\), AbTPO, hypoechoic pattern, and the presence of hypervascularity, only age and TSH were significantly related to serum CXCL10 levels (Table 2).

Patients with AT and hypothyroidism had lower thyroid volume (9 ± 6 vs. 16 ± 14 ml; \( P = 0.02 \)), FT\(_4\) [7.7 ± 2.0 vs. 9.4 ± 2.0 ng/liter (9.9 ± 2.6 vs. 12.1 ± 2.6 pmol/liter); \( P = 0.007 \)], and FT\(_3\) [3.3 ± 0.5 vs. 3.6 ± 0.5 pg/ml (5.1 ± 0.77 vs. 5.5 ± 0.77 pmol/liter); \( P = 0.04 \)] levels and higher degrees of echogenicity (0.9 ± 0.2 vs. 0.6 ± 0.5 \( \text{SD} \) score units; \( P = 0.001 \)), AbTPO titers (495 ± 394 vs. 283 ± 342 IU/ml; \( P = 0.004 \)), and CXCL10 (Fig. 1) than nonhypothyroid patients.

By defining a high CXCL10 level as a value at least 2 \( \text{SD} \) above the mean value of the control group, 31% of patients with AT, 6% of controls, and none of the multinodular goiter patients had high CXCL10 (\( P < 0.0001 \)). In AT patients, high CXCL10 levels were significantly associated with age (51 ± 16 vs. 44 ± 13; \( P = 0.0004 \)), but not with thyroid volume or TSH, FT\(_3\), FT\(_4\), AbTg, or AbTPO levels.

**TABLE 2.** Multiple linear regression of CXCL10 in euthyroid or hypothyroid patients with AT or multinodular goiter.

<table>
<thead>
<tr>
<th></th>
<th>Regression coefficient (R.C.)</th>
<th>CI (95% lower)</th>
<th>CI (95% upper)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>0.39</td>
<td>0.01</td>
<td>0.00</td>
<td>0.02</td>
</tr>
<tr>
<td>TSH (ln[U/ml])</td>
<td>0.41</td>
<td>0.23</td>
<td>0.11</td>
<td>0.35</td>
</tr>
<tr>
<td>Thyroid volume (ml)</td>
<td>0.03</td>
<td>0.00</td>
<td>-0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>AbTPO (IU/ml)</td>
<td>-0.01</td>
<td>-0.00</td>
<td>-0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>FT(_4) (ng/liter)</td>
<td>0.06</td>
<td>0.02</td>
<td>-0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Hypoechoicinity (AU)</td>
<td>0.23</td>
<td>0.29</td>
<td>-0.02</td>
<td>0.61</td>
</tr>
<tr>
<td>Hypervascular pattern (AU)</td>
<td>-0.15</td>
<td>-0.21</td>
<td>-0.51</td>
<td>0.08</td>
</tr>
</tbody>
</table>

CI, Confidence interval; AU, arbitrary units; R.C., regression coefficient. Serum TSH, U/ml = mU/liter; conversion factor for FT\(_4\) = 1.286.

**Discussion**

The AT patients in the present study were typical with respect to clinical phenotype and pattern of autoimmune markers. In general, the thyroid glands of the AT patients were hypothyroid and dysthymogeneous, the more so the higher the autoantibody titer. Thyroid volume was larger in the patients with multinodular goiter; in contrast, thyroid volume was not significantly different between AT patients and controls. The slightly elevated TSH levels in the AT group was due to the inclusion of some hypothyroid patients.

Circulating CXCL10 levels were clearly elevated in AT patients compared with normal controls or patients with multinodular goiter; within the AT group, higher CXCL10 levels were associated with older age, a hypoechoic gland, and hypothyroidism. In hypothyroid patients with AT, CXCL10 levels were more strongly associated with the hypothyroidism itself than with other well known parameters, such as thyroid volume or AbTPO levels. Furthermore, CXCL10 levels were correlated with age and serum TSH concentrations in AT patients as a whole. Overall, these results imply that elevated CXCL10 is not only associated with the autoimmune process itself, but may be a marker of an aggressive thyroditic process eventually leading to the destruction of thyroid follicular cells with the attendant impairment in thyroid function.

AT is the most common and extensively studied organ-specific autoimmune disorder in humans. The mechanisms responsible for initiating thyroid autoimmunity and promoting the progression of the disease remain partially unknown (23). As reported in several human autoimmune conditions, CD4 T lymphocytes play a central role in the induction of the autoimmune response and as effectors of tissue damage (24). Despite extensive research, the multistep process of lymphocyte recruitment, in situ maintenance, and the subsequent disruption of thyroid follicular structure is still controversial. Recent observations have indicated that specific combinations of different inflammatory cytokines (interferon-\( \gamma \) and/or TNF-\( \alpha \)) transform nondestructive into destructive thyroiditis in murine experimental AT (25, 26). Furthermore, it has been suggested that one of the differences between murine experimental AT and human AT is that human thyroid glands show a chronic inflammatory environment enriched mainly with Th1 cytokines such as interferon-\( \gamma \) and TNF-\( \alpha \) and enhanced apoptosis (25, 27). The elevated levels of circulating CXCL10 in patients with AT may result from secretion by both lymphocytes and thyroid follicular cells modulated through interferon-\( \gamma \) (7–9, 28). CXCL10-induced recruitment of Th1 lymphocytes and/or TNF-\( \alpha \) transform nondestructive into destructive thyroiditis in murine experimental AT (25, 26). Furthermore, it has been suggested that one of the differences between murine experimental AT and human AT is that human thyroid glands show a chronic inflammatory environment enriched mainly with Th1 cytokines such as interferon-\( \gamma \) and TNF-\( \alpha \) and enhanced apoptosis (25, 27). The elevated levels of circulating CXCL10 in patients with AT may result from secretion by both lymphocytes and thyroid follicular cells modulated through interferon-\( \gamma \) (7–9, 28). CXCL10-induced recruitment of Th1 lymphocytes and/or TNF-\( \alpha \) transform nondestructive into destructive thyroiditis in murine experimental AT (25, 26).
phocytes, which secrete interferon-γ, in turn stimulates chemokine production by follicular cells, thus maintaining the autoimmune process (11, 28).

The association of high CXCL10 levels with a hypoechogenic pattern of the thyroid gland in AT patients is readily explained by the presence of a marked lymphocyte infiltration, the histological hallmark of AT. This is in agreement with previous reports showing that CXCL10 plays a role in massive T cell infiltrates in the liver (29). In our patients, a hypoechogenic pattern was strongly associated with thyroid dysfunction (14, 19); because serum CXCL10 concentrations were higher in hypothyroid than euthyroid patients independently of hypoechogenicity, it is possible that CXCL10 indicates a stronger inflammatory response, resulting in more extensive tissue destruction. The above-cited experimental data (25, 26) support this hypothesis. In fact, CXCL10 displays a strong chemoattractant activity for Th1 lymphocytes secreting interferon-γ whose intraglandular production results in thyrocyte apoptosis (25) and severe hypothyroidism (26). Accordingly, the recent observation that high pretransplant serum CXCL10 levels predict severe acute rejection and allograft failure in patients undergoing renal transplantation strengthens the hypothesis that CXCL10 measurement represents a reliable marker of aggressive Th1-mediated autoimmune disease (30).

Identification of markers of thyroid destruction represents the first step in the development of preventive therapeutic strategies that could obviate the need for costly levothyroxine replacement therapy and subsequent biochemical monitoring of serum thyroid hormones. Whether circulating CXCL10 levels may serve this purpose requires longitudinal observations in large patient cohorts.

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