Graves' ophthalmopathy: in search of better markers and better treatment
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Chapter 3

Serum IL-18 levels are not increased in patients with untreated Graves’ ophthalmopathy.

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Cytokines play an important role in autoimmune thyroid diseases, and serum levels may reflect the activity of the immune process. This is particularly interesting in Graves' ophthalmopathy, where a reliable serum activity marker is warranted. Interleukin-18 (IL-18) is a potent Th1 cytokine, known to induce interferon (IFN)-gamma and the aim of this study was to evaluate serum IL-18 levels in Graves' ophthalmopathy.

Serum IL-18 was measured by ELISA in 52 patients with untreated Graves' ophthalmopathy (who all had been rendered euthyroid with antithyroid drugs), 52 healthy controls matched for sex, age, and smoking habits and 15 euthyroid patients who had been treated for Graves' hyperthyroidism and ophthalmopathy in the past.

Serum IL-18 (median values in pg/ml with range) levels did not differ between the untreated Graves' ophthalmopathy patients [226 (61-704)], matched healthy controls [194 (17-802)], and Graves' ophthalmopathy patients who were treated in the past [146 (0-608)]. No correlation was observed between serum IL-18 levels and thyroid function or antithyroid antibodies. There was no correlation between serum IL-18 levels and smoking habits.

We conclude that Graves' ophthalmopathy does not affect serum IL-18.

Introduction

Interleukin-18 (IL-18) is a very potent cytokine with pleiotropic activities. Its precursor, Pro-IL-18 can be produced by a variety of cell types, such as monocytes/macrophages, dendritic cells, Kupffer cells, synovial fibroblasts, keratinocytes and osteoblasts. Pro-IL-18 is cleaved by Interleukin-1β converting enzyme to yield the active 18 kDa glycoprotein IL-18. IL-18 is mainly produced by antigen presenting cells, and induces IFN-γ production in synergy with IL-12 which leads to Th1 differentiation. It also exerts pro-inflammatory properties by enhancing the production of IL-1β, Tumour Necrosis Factor alpha (TNFα), chemokines, nitric oxide and prostaglandins.

The cytokine network plays an important role in inflammatory conditions and in autoimmune diseases. During an autoimmune attack, it has been suggested that there is a change in the balance between autoreactive and regulatory cell types, and thus in Th1 and Th2 cell-derived cytokines. In synovial tissues of rheumatoid arthritis patients, both IL-18 mRNA and protein levels were found to be higher than in controls. Although cytokines are produced in the inflamed tissues only, some could be found in high levels in serum. Studies in different fields have found changes in serum IL-18 levels, for instance elevated serum IL-18 levels in schizophrenia as compared to age- and sex-matched controls, and higher levels of IL-18 in biliary atresia.

One study showed serum IL-18 levels to be elevated in Multiple Sclerosis and moreover that serum IL-18 levels correlate with disease activity as measured by MRI.

Increased serum IL-18 levels were reported in untreated Graves' hyperthyroid patients as compared to healthy controls, but not in patients with Hashimoto's thyroiditis. As far as we know, no studies have been published evaluating serum IL-18 levels in Graves' ophthalmopathy patients.

Serum levels of cytokines might be potentially important in making the distinction between active and inactive disease, as the former but not the latter might be susceptible to immunosuppressive therapy. In GO, serum levels of soluble IL-2 receptor, IL-6
and IL-6 receptor are elevated. Additionally, IL-1 and IL-1 receptor antagonist levels increase in response to radiotherapy and IL-4 and IL-10 in response to methylprednisolone. A combination of IL-6, soluble CD30 and soluble TNFα receptor 1 also had some value in predicting response to radiotherapy. However, none of these can be used in clinical practice as a surrogate disease activity parameter because of lack of a good positive/negative predictive value.

We were interested to find out whether serum IL-18 levels were changed in Graves' ophthalmopathy patients, and whether or not this is due to thyroid disease or the eye disease itself. We therefore decided to measure serum IL-18 levels in 52 untreated, euthyroid Graves' ophthalmopathy patients and 52 healthy controls, matched for age, sex and smoking habits and in 15 euthyroid patients who were treated for ophthalmopathy and Graves' hyperthyroidism in the past.

**Patients and methods**

**Patients** We studied 52 patients with untreated Graves' ophthalmopathy who had been rendered euthyroid mostly by antithyroid drugs and 52 controls, matched for age, sex and smoking habits. Patients were enrolled between 1996 and 2000 and suffered from mild (n=18), moderately severe (n=23) or very severe (n=11) eye disease; serum samples were available. Controls were recruited from the general population by advertisement to participate in a study to determine reference values. We additionally studied 15 patients who had been treated for Graves' hyperthyroidism and ophthalmopathy (ex GO patients) (mean ±SD) 13.8 ± 3.6 years before and did not receive current treatment for either disease. Neither patients nor controls were affected with diseases known to influence serum IL-18 levels. Smokers were defined as individuals who currently smoked cigarettes and non-smokers as those who did not.

**Eye measurements** The severity of the eye involvement varied from mild to very severe and was graded using the Total Eye Score. The Total Eye Score was calculated as the sum of each NOSPECS class present times the grade in that class (for that purpose we substituted 1, 2 and 3, respectively, for grades a, b, and c). Ophthalmopathy activity was scored using the Clinical Activity Score based upon classic inflammation parameters: rubor, dolor, tumour and functio laesa.

**Methods** Serum samples were stored at -20°C, until use. All samples were measured in duplicate. A highly sensitive, commercially available ELISA kit (Quantikine, R&D Systems, Minneapolis, MN) was used to measure serum concentrations of IL-18 (detection limit 12 pg/ml, CV 10%). Free T₄ (fT₄) was determined with either a coated tube ¹²²I radioimmunoassay (SPAC, Byk-Sangtec Diagnostica, Dietzenbach 2, Germany), or a solid-phase time-resolved fluoroimmunoassay (Delfia, Wallac Oy, Turku, Finland). TSH was measured in a chemiluminescent enzyme immunoassay (Immulite Third Generation TSH kit, DPC, Los Angeles). Thyrotropin receptor antibodies were measured as TSH Binding Inhibitory Immunoglobulins (TBII) in the TRAK assay (BRAHMS Diagnostica, Berlin, Germany).

**Statistical analysis** With an SD of 45 and a mean of 126 pg/ml IL-18 concentration in healthy controls (according to the manufacturer) and an expected difference of 28 pg/ml for Graves ophthalmopathy patients, we calculated a sample size of 55 patients (α error 0.05, power 0.64). To analyse differences between two groups we used the Mann-Whitney U-test. We used the Wilcoxon rank test for paired data where appropriate. Correlations were calculated with the Spearman correlation coefficient.
Results

The results are presented in Table 1 and Figure 1. Serum IL-18 (median values in pg/ml with range) levels did not differ between 52 euthyroid, untreated Graves’ ophthalmopathy patients [226 (61–704) pg/ml], 52 matched, healthy controls [194 (17–802) pg/ml] and 15 euthyroid patients treated for Graves’ hyperthyroidism and ophthalmopathy in the past [146 (0–608) pg/ml].

There was no significant correlation between serum IL-18 levels and age, sex, smoking habits or thyroid function (TSH and fT₃). There were 41 TBI levels from 52 patients within the group of Graves’ ophthalmopathy patients. A positive TBI level was defined as higher than 12 U/l, so 23/41 patients had a median TBI of 36 U/l, range 13-405. From 15 patients who were treated for Graves’ hyperthyroidism and ophthalmopathy in the past only two had a positive TBI (16 and 78, respectively). There was no significant correlation between IL-18 and TBI antibodies. There was no correlation between serum IL-18 levels and activity or severity of the eye disease as measured by the Clinical Activity Score and Total Eye Score within the group of eye-disease patients. There was also no correlation with smoking habits.

Discussion

We did not observe any correlation between serum IL-18 levels and TSH, fT₃, or TBI in our euthyroid Graves’ ophthalmopathy patients. Miyauchi et al.² reported higher serum IL-18 levels in Graves’ hyperthyroidism patients in the hyperthyroid state as compared to the euthyroid state, but also failed to find any correlation between serum IL-18 levels, TBI titers, fT₃, and freeT₄. We did not observe that serum levels of IL-18 were related to activity or severity of Graves’ ophthalmopathy. Serum IL-18 measurements therefore cannot be used as a serum marker of Graves’ ophthalmopathy in clinical practice.

It is still possible that IL-18 plays a local role in the orbit. IL-18 is mainly a Th1 cytokine and GO has been reported to be a Th1 rather than a Th2 type disease.¹⁵ It may very well be that serum levels do not reflect the local tissue concentrations. In addition, in a previous study, we found similar levels of IL-18 mRNA in orbital connective tissues from patients with active vs. inactive eye disease, making it less likely that IL-18 is a marker of activity in Graves’ ophthalmopathy.¹⁶ In these orbital connective tissue samples of patients with active and severe untreated GO, we found higher mRNA levels of inflammatory cytokines (IL-1, IL-6, IL-8 and IL-10) and higher IL-2, as a marker of a predominantly Th1 profile compared with tissues from inactive GO patients.

In earlier studies we found that serum concentrations of a variety of cytokines and adhesion molecules, like IL-1 receptor

Figure 1: Median and individual serum IL-18 levels in 52 untreated euthyroid Graves’ ophthalmopathy patients (untreated GO), 52 healthy controls matched for sex, age and smoking habits and 15 patients who were treated more than 10 years ago for Graves’ hyperthyroidism and GO (ex GO). Statistical analysis was performed with the Mann Whitney U-test.
Table 1: Characteristics and thyroid function of 52 untreated Graves’ ophthalmopathy patients (GO), 52 healthy sex and age matched controls and 15 patients who were treated more than 10 years ago for Graves’ hyperthyroidism and ophthalmopathy (ex GO)

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>GO</th>
<th>ex GO</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=52</td>
<td>n=52</td>
<td>n=15</td>
<td></td>
</tr>
<tr>
<td>F/M</td>
<td>41/11</td>
<td>41/11</td>
<td>9/6</td>
</tr>
<tr>
<td>Age (yrs ±SD)</td>
<td>52 ± 9.5</td>
<td>52 ± 9.5</td>
<td>58 ± 9</td>
</tr>
<tr>
<td>Smoking yes/no</td>
<td>27/25</td>
<td>27/25</td>
<td>8/7</td>
</tr>
<tr>
<td>Duration eye disease in months median/range</td>
<td>-</td>
<td>13/1–156</td>
<td>150/102–276</td>
</tr>
<tr>
<td>Clinical Activity Score (0–10)* median/range</td>
<td>-</td>
<td>4/0–9</td>
<td>0/0</td>
</tr>
<tr>
<td>Total Eye Score (0–60)* median/range</td>
<td>-</td>
<td>12/4–36</td>
<td>6/0–20</td>
</tr>
<tr>
<td>TSH (0.4–4 mU/l)* mean ±SD</td>
<td>2.0 ± 1.4</td>
<td>1.8 ± 1.9</td>
<td>1.0 ±1.0</td>
</tr>
<tr>
<td>fT₄ (10–23 pmol/l)* mean ±SD</td>
<td>14 ± 2</td>
<td>16± 4</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>TBII (positive if &gt;12 U/l) n positive/ n total</td>
<td>-</td>
<td>23/41</td>
<td>2/15</td>
</tr>
<tr>
<td>IL18 pg/ml median/range</td>
<td>194/17–802</td>
<td>226/61–704</td>
<td>146/0–608</td>
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</table>

* Normal value

antagonist, IL-6 receptor and sICAM-1 are higher in smokers than non-smokers, perhaps reflecting an overall upregulation of the activity of the immune system. This may be of importance in Graves’ ophthalmopathy because smoking is a strong risk factor for its development. Interestingly, serum IL-18 levels were not found to be influenced by smoking. From our observations we conclude that serum IL-18 levels are not correlated to either the activity or the severity of Graves’ ophthalmopathy.

References


