Serial analysis of the effects of methimazole or radical therapy on circulating CD16/56 subpopulations in Graves' disease

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Abstract
The distribution of peripheral blood CD16/56 cytotoxic T and natural killer (NK) cells in Graves’ disease patients is analyzed in order to correlate them with disease activity and with prognosis. Eighteen patients with Graves’ disease, twenty-four patients with Hashimoto’s thyroiditis and thirty-two sex- and age-matched healthy control subjects were studied. Peripheral blood CD16/56 (cytotoxic T and NK) cells were analyzed by cytofluorometry. A decreased proportion of CD16+/56+ and CD16/56+CD3+ cells were detected in Graves’ disease patients when compared with thyroiditis patients and healthy control groups. No correlation was detected with serum free thyroxine. On diagnosis, patients who would require a radical treatment for thyrotoxicosis control showed a significant decrease of cytotoxic CD56+ T (CD3+) and NK (CD3−) cells compared with those who would maintain the euthyroid state after methimazole. These results suggest that the cytotoxic compartment, both T and NK cells, of the immune system is altered in patients with Graves’ disease, independently of the functional thyroid status. Changes in peripheral blood lymphocytes in Graves’ disease patients could be useful as predictive markers of an unfavorable outcome.

European Journal of Endocrinology 139 314–316

Introduction
Graves’ disease (GD) is a chronic disease of unknown etiology, characterized by reactivity to self thyroid antigens (1). Several abnormalities of the immune system have been described in GD patients, either within the thyroid gland (2, 3) or in the peripheral blood (4–12). CD16/56+ cells have rarely been analyzed in GD patients (10–13). A decreased percentage of CD56+ cells (12) as well as a diminished natural killer (NK) activity in peripheral blood from hyperthyroid GD patients (12) has been demonstrated. However, to our knowledge, a longitudinal study of changes in CD16/56 population subsets has not been performed until now. We have investigated the quantitative abnormalities of peripheral blood CD16/56+ cells in a clinically homogeneous group of untreated patients with GD in order to correlate them with disease activity. Likewise, the predictive value of quantitative alterations in lymphocyte subpopulations was considered.

Patients and methods
Subjects
We studied 18 patients with GD, 24 patients with Hashimoto’s thyroiditis (HT) and 32 sex- and age-matched healthy control subjects (Table 1). GD and HT patients were enrolled consecutively from those attending our outpatient clinic. All patients were untreated at the start of the study. The diagnosis of GD and HT was based on accepted criteria (14). To be accepted in the experimental protocol, the patients had to comply with the following conditions: (i) absence of clinical evidence of disease, other than thyroid autoimmune disease and (ii) absence of previous or actual treatment. Patients and controls gave informed consent for inclusion in the study.

Immunological marker analysis
Peripheral blood samples were analyzed by direct immunofluorescence, as previously described (15). Monoclonal antibodies directed against membrane antigens were employed to characterize the following subpopulations: anti-CD3 (pan-T) and anti-CD16/56 (pan-NK) (Becton-Dickinson, Mountain View, CA, USA).

Thyroid hormones and autoantibodies
Serum free thyroxine levels (FT4), serum free triiodothyronine levels (FT3) and thyrotropin (TSH) were measured with radioimmunoassay kits from Diagnostic Products Corporation (Los Angeles, CA, USA). The serum titers of anti-TSH receptor (TBII) antibodies, anti-thyroid...
radical therapy, with euthyroidism achieved in every case. These patients were also re-evaluated three months after the first visit. Then, GD patients were treated with methimazole, 15 mg/day, until euthyroidism was achieved. Levothyroxine was added if hypothyroidism appeared. Patients were followed-up for 24 months after the diagnosis. Fourteen patients achieved control of hyperthyroidism by receiving methimazole for 12 months and remained euthyroid after the cessation of treatment (GD patients with favorable outcome). At the 12th week, when a euthyroid state had been obtained, lymphocyte subpopulations were re-evaluated. The rest of the patients (4 cases) required radical treatments (¹¹¹I in three cases; surgery in one case) because of clinical and analytical manifestations of hyperthyroidism despite anti-thyroid therapy (GD patients with unfavorable outcome). These patients were also re-evaluated three months after radical therapy, with euthyroidism achieved in every case.

Study schedule

Analysis of lymphocyte markers was performed on the first visit. Then, GD patients were treated with methimazole, 15 mg/day, until euthyroidism was achieved. Levothyroxine was added if hypothyroidism appeared.

Patients were followed-up for 24 months after the diagnosis. Fourteen patients achieved control of hyperthyroidism by receiving methimazole for 12 months and remained euthyroid after the cessation of treatment (GD patients with favorable outcome). At the 12th week, when a euthyroid state had been obtained, lymphocyte subpopulations were re-evaluated. The rest of the patients (4 cases) required radical treatments (¹¹¹I in three cases; surgery in one case) because of clinical and analytical manifestations of hyperthyroidism despite anti-thyroid therapy (GD patients with unfavorable outcome). These patients were also re-evaluated three months after radical therapy, with euthyroidism achieved in every case.

Statistical analysis

The data from the groups were compared with the Mann-Whitney U test. Significances of parameters within each group were tested by the Wilcoxon matched-pairs signed rank test. For correlation studies, linear-regression analysis by the least squares method was used. A P value less than 0.05 was considered to indicate a significant difference.

Table 1 Characteristics of healthy controls and patients with Graves’ disease or Hashimoto’s thyroiditis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal range</th>
<th>Healthy controls</th>
<th>Hashimoto’s thyroiditis patients</th>
<th>Graves’ disease patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.1 ± 14.5</td>
<td>36.6 ± 16.9</td>
<td>33.8 ± 15.2</td>
<td></td>
</tr>
<tr>
<td>Male:female ratio</td>
<td>1:16</td>
<td>0:24</td>
<td>1:8</td>
<td></td>
</tr>
<tr>
<td>No. of patients with goiter (%)</td>
<td>–</td>
<td>19 (79.1)</td>
<td>18 (100)</td>
<td></td>
</tr>
<tr>
<td>No. of patients with infiltrative ophthalmopathy (%)</td>
<td>–</td>
<td>–</td>
<td>6 (37.5)</td>
<td></td>
</tr>
<tr>
<td>Free T4 (pmol/l)</td>
<td>11.4–23.8</td>
<td>16.2 ± 4.3</td>
<td>15.6 ± 7.1</td>
<td>57.1 ± 16.5</td>
</tr>
<tr>
<td>Free T3 (pmol/l)</td>
<td>3.0–8.0</td>
<td>4.4 ± 1.3</td>
<td>3.4 ± 0.9</td>
<td>19.9 ± 13.4</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>0.4–3.1</td>
<td>1.8 ± 0.7</td>
<td>21.4 ± 34.0</td>
<td>0.07 ± 0.09</td>
</tr>
<tr>
<td>Anti-TSH receptor antibodies (IU/ml)</td>
<td>&lt;14</td>
<td>0</td>
<td>7.8 ± 6.7</td>
<td>85.7 ± 65.2</td>
</tr>
<tr>
<td>Anti-thyroglobulin antibodies (IU/ml)</td>
<td>&lt;130</td>
<td>0</td>
<td>3153.0 ± 2287.1</td>
<td>2091.9 ± 2416.5</td>
</tr>
<tr>
<td>Antithyroid peroxidase antibodies (IU/ml)</td>
<td>&lt;200</td>
<td>0</td>
<td>985.8 ± 1352.2</td>
<td>425.5 ± 551.0</td>
</tr>
</tbody>
</table>

Table 2 Lymphocytes and CD16/56 lymphocyte subpopulations in healthy controls, Hashimoto’s thyroiditis patients and Graves’ disease patients with favorable or unfavorable outcome.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy controls (n = 32)</th>
<th>Hashimoto’s thyroiditis patients (n = 24)</th>
<th>Graves’ disease patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes (cells/mm³)</td>
<td>2103 ± 415</td>
<td>1965 ± 574</td>
<td>2354 ± 857</td>
</tr>
<tr>
<td>CD3⁺ (%)</td>
<td>65.2 ± 8.3</td>
<td>68.1 ± 8.1</td>
<td>68.7 ± 9.1</td>
</tr>
<tr>
<td>CD16/56⁺ (%)</td>
<td>29.8 ± 12.1</td>
<td>21.2 ± 8.7</td>
<td>16.3 ± 9.1a</td>
</tr>
<tr>
<td>CD16/56⁺CD3⁻ (%)</td>
<td>16.5 ± 8.7</td>
<td>15.6 ± 8.8</td>
<td>13.5 ± 8.2</td>
</tr>
<tr>
<td>CD16/56⁺CD3⁺ (%)</td>
<td>13.1 ± 5.3</td>
<td>6.7 ± 5.9</td>
<td>4.2 ± 3.1b</td>
</tr>
</tbody>
</table>

a P < 0.01 vs healthy controls and Hashimoto’s thyroiditis patients; b P < 0.01 vs healthy controls and P < 0.05 vs Hashimoto’s thyroiditis patients; c P < 0.05 vs Graves’ disease patients with favorable outcome.
After radical treatment, GD patients showed an increase in the percentage of CD16/56+ cells (from 9.52 ± 3.93% to 14.62 ± 5.42%), the statistically significant differences disappearing when compared with healthy controls. However, the methimazole-induced euthyroidism (GD patients with a favorable outcome) did not modify the altered percentages of CD16/56+ cells observed on diagnosis (from 18.3 ± 9.3% to 17.1 ± 8.5%).

**Discussion**

Although GD is a condition characterized by an intrathyroidal lymphocytic infiltration (1), our studies were performed on peripheral blood. Nevertheless, the clinical, biochemical and immunological disturbances in GD are not limited solely to the thyroid gland (1, 16). Moreover, data from surgically obtained thyroid tissue are representative of that group of GD patients with an unfavorable prognosis, but are not representative of the GD patients overall.

As reported by Lanier et al. (17), within the CD16/56+ cell population, there are two subsets: CD16/56+CD3+ (cytotoxic T cells) and CD16/56+CD3− (NK cells). Our study has demonstrated a decreased number of both CD16/56+ cell subsets. Although the decreased cytolytic activity has been related to hyperthyroxinemia (10), our results do not agree with this hypothesis: the CD16/56+ cell percentage continued to be decreased in methimazole-treated patients. By contrast, the near or total normalization of altered CD16/56+ cell subsets percentages in GD patients who underwent a radical therapy was remarkable. These results indicate the need for a chronic antigenic stimulation to maintain the modifications observed in the cytotoxic compartment of the immune system. Although our results are handicapped by the relatively small number of patients with an unfavorable outcome, we show that the decrease of CD16/56+ cells could be used as a prognostic marker of GD. The decreased potential capability of performing the cytotoxic function (decrease of CD16/56+ cells) is concordant with the outcome of GD: antigen recognition is not followed by the destruction of follicular cells, but by the induction of hyperthyroidism by autoantibodies directed against TSH receptors (1, 12, 16).

**Acknowledgements**

This work was supported by a grant from INSALUD (FIS 93/1318).

**References**

4. Karlsson EA & Totttenm H. Immunomodulation by methimazole therapy in Graves’ disease: rapid changes in activation stage of circulating regulatory T cell subsets, B cells and NK cells. *Clinical and Experimental Immunology* 1988 74: 258–263.

Received 6 January 1998
Accepted 27 April 1998