Thyroid-infiltrating lymphocytes, thyroid function, and HLA-DR in juvenile autoimmune thyroiditis

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ABSTRACT. Eighteen patients with juvenile autoimmune thyroiditis were studied. At diagnosis 8 (44%) of the patients were euthyroid and 10 hypothyroid, whereas at the end of 6 to 12 months follow-up, 12 (66%) were euthyroid and 6 hypothyroid. All the patients were HLA-typed. The frequency of HLA-DR4 was increased in the patients when compared with the normal population, 63 vs 28% (p < 0.01). An analysis of thyroid-infiltrating mononuclear cells revealed that the majority of the thyroid-infiltrating lymphocytes were T cells. More T lymphocytes and fewer B lymphocytes and HLA class II positive lymphocytes were found among the thyroid-infiltrating mononuclear cells in euthyroid than hypothyroid patients. The numbers of thyroid-infiltrating B lymphocytes correlated with the levels of thyroid microsomal antibodies. No correlation was found between thyroid function and thyroid antibodies.

Although juvenile autoimmune thyroiditis, the most common thyroid disease in childhood and adolescence (1,2), was thought always to lead to hypothyroidism (3–5), follow-up studies have revealed that half of the patients remain euthyroid, one third develop overt hypothyroidism, and the rest are subclinically hypothyroid (6,7). In some patients the thyroid function may fluctuate between hyper- and hypothyroidism (6–8). In individual patients serology at presentation or later correlates poorly with the thyroid function or changes in it (9–11).

To test whether the cellular composition of the mononuclear cell infiltrate in the thyroid, or the proportion of lymphocytes expressing class II HLA antigens (i.e. activated) in the infiltrate, correlates with thyroid function, we assessed the proportions of T and B cells and lymphocytes expressing HLA class II among the thyroid-infiltrating cells from fine needle aspiration biopsies of the thyroid and compared them with thyroid function and thyroid antibodies. The patients were also HLA-typed to test whether the frequencies of HLA-DR antigens, reported to be altered in patients with thyroid dysfunction (12–15), differed from those in the normal Finnish population. We report here the results of such a study.

Patients and Methods

Patients

Eighteen patients (14 females, 4 males, mean age 13.5 ± 2.1 years, range 9.4–16.5) with a history of thyroid disease of 1.0 ± 0.9 (range 0.1–3.0) years were tested at diagnosis and 6 and/or 12 months later. The thyroid glands were moderately enlarged in all patients but one, a 9-year-old girl with a small firm thyroid, retarded growth, and other signs of hypothyroidism. All the hypothyroid patients were treated with thyroxine. Informed consent was obtained from the patients and/or their parents and the study was accepted by the Ethical Committee of the Aurora Hospital.

Biochemical assays

Concentrations of serum T4 and TSH were measured using radioimmunoassays (T4-RIA by Beckton-Dickinson, Orangeburg, New York, NY; TSH RIA-gnost hTSH, Behringwerke, Marburg, FRG). Serum free T4 (fT4), T3-
uptake and free T₄ index (FT₄I) were determined as described by Helenius & Liewendung (16). Values considered normal were: T₃, 65–165 nmol/l (<12 years), 65–160 nmol/l (>12 years); FT₄I, 68–153; FT₄, 8–25 pmol/l; TSH, 0.5–6.0 mU/l (<12 years), 0.2–4.0 mU/l (>12 years).

**Fine needle aspiration biopsy**

Fine needle aspiration biopsy (11) was performed using a 0.7 mm outer diameter needle on both thyroid lobes at presentation and then repeated once of twice 6 and/or 12 months later. The patients were given 0.5 mg/kg of diazepam and 0.5 mg/kg of pethidine before aspiration and the skin was anesthetized using 0.25% (w/w) lidocaine cream.

**Characterization of infiltrating cells**

A three-layer immunoperoxidase technique described in detail elsewhere (11) was used to identify the cells in the cytospun preparations of the infiltrate. The monoclonal antibodies used were OKT11, OKT4 and OKT8 (Ortho Pharmaceutical Co, Raritan, NJ) staining all T (CD2) cells, T cells of the helper/inducer (CD4) and of the suppressor/cytotoxic subset (CD8), respectively, and B1 (Coulter Immunology, Hialeah, FL) staining B cells. Lymphoid cells expressing HLA class II antigens were detected by staining with OKIa1 (Ortho). B cells were also stained using anti-kappa and anti-lambda antibodies (Beckton-Dickinson, Mountain View, CA).

**Serology**

Thyroglobulin and thyroid microsomal antibodies were assayed using passive haemagglutination (Thymune-T and -M tests, Wellcome Diagnostics, London, UK). Titres ≥6400 (detectable in <5% of the normal population) were considered positive.

**HLA-DR-typing**

HLA-DR-typing was performed at the Finnish Red Cross Blood Transfusion Service using antibody-mediated cytotoxicity as described previously (17). The frequencies of HLA-DR antigens in the normal population were obtained by testing 322 normal blood donors.

**Statistics**

Fisher’s exact probability test, ANOVA, Student’s paired t-test, and the Wilcoxon’s rank sum test were used.

**Results**

**Thyroid function**

On the basis of T₃, FT₄I, FT₄, and TSH levels 8 of the 18 patients (44%) were euthyroid, 5 (28%) subclinically hypothyroid (T₃, FT₄I and FT₄ within normal range and TSH above the normal range), and 5 (28%) hypothyroid at presentation. In 13 of the 18 patients (72%) the thyroid function did not change during the follow-up. In 3 subclinically hypothyroid (TSH 7.3–15.9 mU/l) patients thyroid function returned to normal during the follow-up, in one subclinically hypothyroid patient clinical hypothyroidism developed, and one patient, hypothyroid at presentation (T₄ 48 nmol/l, FT₄I 42, TSH 24.0 mU/I), was hyperthyroid (FT₄ 27 pmol/l, no TSH-response to TRH stimulation) 7 months and euthyroid 11 months later. Thus, at the end of the follow-up 12 of the patients (66%) were euthyroid and 6 (34%) hypothyroid (one subclinically).

**Table 1.**

<table>
<thead>
<tr>
<th>Antibody used to stain lymphocytes</th>
<th>Cell defined</th>
<th>Follow-up</th>
<th>At presentation</th>
<th>At the end of study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Euthyroid</td>
<td>Hypothyroid</td>
<td>Euthyroid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(N = 8)</td>
<td>(N = 10)</td>
<td>(N = 12)</td>
</tr>
<tr>
<td>OKT11</td>
<td>CD2</td>
<td>65.8 ± 6.3</td>
<td>58.3 ± 11.7</td>
<td>67.5 ± 7.2</td>
</tr>
<tr>
<td>OKT 8</td>
<td>CD8</td>
<td>26.6 ± 7.1</td>
<td>22.4 ± 4.8</td>
<td>25.3 ± 5.3</td>
</tr>
<tr>
<td>OKT 4</td>
<td>CD4</td>
<td>52.4 ± 9.9</td>
<td>49.4 ± 12.0</td>
<td>47.3 ± 10.6</td>
</tr>
<tr>
<td>Kappa lambda</td>
<td>B</td>
<td>20.6 ± 5.7</td>
<td>25.6 ± 11.3</td>
<td>23.6 ± 7.3</td>
</tr>
<tr>
<td>B1</td>
<td>B</td>
<td>24.1 ± 11.1</td>
<td>&lt; 0.05</td>
<td>37.2 ± 6.1</td>
</tr>
<tr>
<td>OKIa1</td>
<td>Class II⁺</td>
<td>30.8 ± 14.9</td>
<td>&lt; 0.01</td>
<td>55.0 ± 14.8</td>
</tr>
</tbody>
</table>

Mean percentages ± so of lymphocytes stained with antibodies indicated given. For p-values Wilcoxon’s rank sum test was used.
Cellular infiltrate of the thyroid

T cells dominated the mononuclear cell infiltrate of the thyroid in both euthyroid and hypothyroid patients. The proportion of T cells was higher in the euthyroid than in the hypothyroid patients (Table 1). Thus, 66–68% of the infiltrating lymphocytes were CD2-positive T cells in the euthyroid and 58–59% in the hypothyroid patients (not significant). The proportions of CD4- and CD8-positive cells were similar in the thyroids of euthyroid and hypothyroid patients. At diagnosis, B lymphocytes were more frequent in the thyroid infiltrate in hypothyroid than in euthyroid patients. Thus, 37% of the lymphocytes infiltrating the thyroid in hypothyroid patients stained with anti-B1 antibody (staining all B lymphocytes) as opposed to 24% in euthyroid patients (p < 0.05). This difference in the numbers of infiltrating B cells was also seen at the end of the follow-up and when anti-kappa and anti-lambda antisera were used to stain the cells, but the differences were statistically not significant. In the thyroid of hypothyroid patients, a higher proportion of the infiltrating lymphocytes was HLA class II positive than in the euthyroid patients. Thus in the beginning of the study, 55% of the thyroid lymphocytes in the hypothyroid patients and 31% in the euthyroid patients were class II positive (p < 0.01), and at the end of the study the percentages were 66 and 43% (p < 0.05), respectively.

In 3 patients the thyroid-infiltrating cells were studied while the thyroid function changed. Proportionally more T cells were detected while thyroid function was normal (70, 85 and 70%) than when the patients were hypothyroid (54, 66 and 58%, respectively). The percentages of CD4-positive cells were 49, 50 and 54 when the thyroid function was normal and 42, 50 and 56 when it was not; those of CD8-positive cells were 21, 33 and 18 and 25, 28 and 20, respectively. The numbers of B cells and HLA class II expressing cells did not change (data not shown).

Fig. 1.

Correlation between log 10 of thyroid microsomal antibody titre and thyroid mononuclear cell staining with (A) anti-kappa and anti-lambda antisera, (B) with anti-B1 antibody.

Table 2.
Frequency of HLA-DR antigens in patients with juvenile autoimmune thyroiditis.

<table>
<thead>
<tr>
<th>DR antigen</th>
<th>Patients</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>3/16 (19)</td>
<td>119/322 (37)</td>
<td>NS</td>
</tr>
<tr>
<td>DR2</td>
<td>3/16 (19)</td>
<td>107/322 (33)</td>
<td>NS</td>
</tr>
<tr>
<td>DR3</td>
<td>6/16 (38)</td>
<td>102/322 (32)</td>
<td>NS</td>
</tr>
<tr>
<td>DR4</td>
<td>10/16 (65)</td>
<td>90/322 (28)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DR5</td>
<td>2/16 (6)</td>
<td>20/322 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>DR6</td>
<td>0/16 (0)</td>
<td>17/322 (5)</td>
<td>NS</td>
</tr>
<tr>
<td>DR7</td>
<td>1/16 (6)</td>
<td>57/322 (18)</td>
<td>NS</td>
</tr>
<tr>
<td>DR8</td>
<td>1/16 (6)</td>
<td>41/322 (13)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Number positive/number tested (%) given.
Thyroid serology compared with the cellular infiltrate of the thyroid

Patients with thyroid microsomal antibodies in titres \( \geq 6400 \) had more B lymphocytes infiltrating the thyroid than had patients with titres \( <6400 \) (Fig. 1). Of the infiltrating lymphocytes 33\% stained with B1 antibody, and 27\% with anti-kappa and anti-lambda antisera in patients with thyroid microsomal titres \( \geq 6400 \) and 24 and 19\%, respectively, in those with titres \( <6400 \) (not significant). A significant correlation was found between the numbers of B lymphocytes stained either with B1 or with anti-kappa and anti-lambda and log 10 microsomal antibody titres \( (r = 0.6, p < 0.05, \text{for both}) \). The titres of thyroid microsomal antibodies did not, however, correlate with thyroid function. Thyroglobulin antibodies in titres \( \geq 6400 \) were detected in only 3 patients.

**HLA-types**

Sixty-three percent (10 of 16) of the patients tested has HLA-DR4 antigen as opposed to 27\% in the normal population \((p < 0.01)\). The frequencies of other HLA-DR antigens did not differ from normal controls (Table 2).

**Discussion**

In children and adolescents juvenile autoimmune thyroiditis is the most common cause of hypothyroidism. An enlarged, firm thyroid gland arouses clinical suspicion of this disorder. The diagnosis can be serologically confirmed in 85\% of the patients, whereas in seronegative patients the findings of small lymphocytes, large macrophages, and atypical follicular epithelium in fine needle aspiration cytology is diagnostic for juvenile autoimmune thyroiditis (9). T cells usually dominate among the thyroid-infiltrating lymphocytes (11, 18–20). The relationship between thyroid function and the subclasses of infiltrating lymphocytes has not been investigated. In the patients described here, the proportion of T cells among the thyroid-infiltrating lymphocytes was higher in euthyroid patients, whereas more B lymphocytes and HLA class II positive lymphocytes were detected in the thyroids of hypothyroid patients. Also, in the 3 patients in whom the thyroid function normalized during the follow-up, the functional change was accompanied by an increase in the numbers of thyroid-infiltrating T cells. In patients with autoimmune thyroiditis and normal thyroid function there were more T cells and fewer B cells and HLA class II positive lymphocytes among thyroid-infiltrating lymphocytes than in hypothyroid patients. T cells infiltrating the thyroid in autoimmune thyroiditis are autoreactive and have been shown to proliferate in response to autologous thyroid cells (21–23). The finding of more class II positive lymphocytes, which include activated T cells and B cells, in the thyroids of hypothyroid patients may suggest a more aggressive autoimmune reaction in these patients.

The finding that at diagnosis about half of the patients were euthyroid and half hypothyroid, together with the change in thyroid function, observed during the follow-up in 28\% of the patients, is in agreement with previous reports (6–8). The change usually observed here was that in patients hypothyroid at presentation, thyroid function returned to normal. As described above, this was accompanied by an increase in thyroid-infiltrating T cells. In agreement with previous studies (6, 9, 10, 24), the titres of thyroid antibodies did not correlate with the thyroid function or changes in it. Patients with higher titres of thyroid antibodies, however, had higher numbers of thyroid-infiltrating B cells, and the numbers of B cells in the thyroid correlated with the titres of microsomal antibodies.

Among the patients with juvenile autoimmune thyroiditis, 63\% had HLA-antigen DR4 compared with 27\% of the normal population \((p < 0.01)\). This association has not, to our knowledge, been shown before. However, other autoimmune thyroid diseases, e.g. Graves' disease, Hashimoto's disease, postpartum and silent thyroiditis, have previously been shown to associate to HLA-DR4, -DR5 or -DR3 (12–15).

The management of thyroid disorders is based on biochemical testing of thyroid function. In seronegative patients fine needle aspiration biopsy is useful in determining the autoimmune nature of the thyroid disease and in differentiation of autoimmune thyroid disease from simple endemic goitre and infectious thyroiditis. An analysis of lymphocyte subclasses and expression of HLA class II on thyroid-infiltrating lymphocytes in samples obtained by fine needle aspiration may support the findings of tests of thyroid function, but do not predict changes in thyroid function. Normal thyroid function seems, however, to be accom-
panied by higher numbers of T cells, fewer B cells, and fewer class II positive lymphocytes in the thyroid infiltrate.

Acknowledgments

We thank Miss Pirjo Toivola, registered nurse, for excellent technical help and cooperation.

References


Received April 3rd, 1989.
Accepted July 10th, 1989.