Altered balance of immunoregulatory T lymphocyte subsets in autoimmune thyroid diseases

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Abstract. Three monoclonal antibodies recognizing cell surface antigens of total peripheral (OKT3), helper/inducer (OKT4) and suppressor/cytotoxic (OKT8) T lymphocytes were used by an indirect immunofluorescence technique to enumerate peripheral T lymphocytes in 25 patients with Graves' disease (including 4 euthyroid Graves' patients), 16 patients with Hashimoto's thyroiditis and 22 normal controls. Total lymphocyte count and percentages of overall T and helper/inducer T cells among peripheral lymphocytes in these conditions showed no significant difference from those of the controls. Percentage of suppressor/cytotoxic T cells, however, was decreased in Graves' disease patients with or without hyperthyroidism. The ratio of helper/inducer T cells to suppressor/cytotoxic T cells was increased in Graves' disease population and slightly increased in hypothyroid Hashimoto's thyroiditis patients. The ratio correlated with the mitogenic response of peripheral mononuclear cells to phytohaemagglutinin, but not with the serum levels of thyroid hormones nor with the titres of thyroid autoantibodies. These findings are in accordance with the results of previous functional studies and indicate possible defects in suppressor T lymphocytes in autoimmune thyroid disease.

Although the pathogenesis of autoimmune disorders has not yet been fully clarified, deficiency in suppressor T lymphocyte function has been postulated as one of the mechanisms involved. As for thyroid autoimmunity, suppressor function of the peripheral lymphocytes has been reported to be decreased in Graves' disease and in Hashimoto's thyroiditis (Aoki et al. 1979; Okita et al. 1981). Recently, a series of murine monoclonal antibodies (OKT) recognizing surface antigens of human T cells was developed. Among these, OKT3 was shown to react with total peripheral T cells, OKT4 with helper/inducer subset of T cells, and OKT8 with suppressor/cytotoxic T lymphocytes (Reinherz & Schlossman 1980). In the present study, we used these three antibodies to enumerate peripheral lymphocytes in autoimmune thyroid diseases and examined whether or not the balance of two major immunoregulatory T cell subsets in these conditions differs from the healthy state.

Materials and Methods

Subjects

Forty-one patients were included in this study. There were 25 with Graves' disease, 5 males and 20 females, aged 17–67 (mean age 38 years). Thirteen of them were hyperthyroid without medication, 4 euthyroid without any treatment and diagnosed by both their eye symptoms and negative response to extrinsic TRH ('so-called' euthyroid Graves' patients), and 8 euthyroid on methimazole. There were 16 patients with Hashimoto's thyroiditis before replacement therapy, 2 males and 14 females, aged 22–73 (mean 53 years). Ten of them were hypothyroid and 6 were euthyroid. Twenty-two healthy adult volunteers without any known endocrine or immunological complication, 16 males and 6 females, aged 22–54 (mean 35 years), were used as normal controls.

Indirect immunofluorescence technique

Peripheral mononuclear cells (PMN) were isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Conray, specific gravity 1.077. After washing the sample three times with RPMI-1640 supplemented with 5% calf serum and 25 mM HEPES, $1 \times 10^6$
cells in 0.2 ml of medium were mixed with 5 µl of one of the OKT monoclonal antibodies (Ortho Pharmaceutical Co., New Jersey, USA) and incubated for 30 min at 4°C. After 2 washes with cold phosphate buffered saline (PBS), cells were incubated with 0.1 ml of an appropriate dilution of fluorescein-conjugated rabbit antimouse immunoglobulin (RAM-FITC) for 30 min at 4°C. After 2 washes with cold PBS, fluorescence positive cells were enumerated by means of a Nikon fluorescent microscope. Assays were done in duplicate and more than 200 PMN for each specimen were counted. Cells treated only with RAM-FITC served as negative controls.

To estimate the proportion of contaminated monocytes in PMN, peroxidase staining (modified McJunkin’s method) was employed. Corrected by this factor, the results were expressed as percentages of fluorescent cells in total lymphocytes. In short, cells reactive with OKT3 will be designated as OKT3+, and the same is the case with OKT4+ and OKT8+.

**Mitogenic response test**

PMN 1 × 10⁵/well were cultured in 0.2 ml of RPMI-1640 supplemented with 15% foetal calf serum with 0.2 µl/well of phytohaemagglutinin (PHA-P, Difco Laboratories, Michigan, USA) at 37°C. After 48 h of incubation, 0.25 µCi of [³H]thymidine was added to each well and cells were harvested 20 h later. The radioactivity on glass fibre filters was counted in a Packard liquid scintillation counter.

**TSH-binding inhibitor immunoglobulins (TBII)**

The TBII activity of sera was assessed by the radioreceptor assay of TSH using the Triton-solubilized human thyroid receptors as previously reported (Iida et al. 1982).

**Antithyroid antibodies**

Titres of antimicrosomal and antithyroglobulin antibodies were measured by tanned red cell agglutination with commercial kits (Fujizoki Pharmaceutical Co., Tokyo, Japan).

**Statistics**

All data were analysed for statistical significance by Student’s t-test.

**Results**

The peripheral lymphocyte count and the percentages of cells reactive with the monoclonal antibodies in autoimmune thyroid diseases are shown in Table 1. The lymphocyte count and percentages of OKT3+ and OKT4+ in the patients were comparable to those in the control subjects. The percentage of OKT8+ was significantly decreased in Graves’ patients, both in hyperthyroid and in euthyroid conditions.

Fig. 1 shows the ratio of OKT4+/OKT8+ (T4+/T8+), which represents the balance of two major immunoregulatory T cell subsets. Three groups of patients, i.e. Graves’ patients with hyperthyroidism and in euthyroid state with or without antithyroid drug treatment, had higher T4+/T8+ than controls. Hypothyroid Hashimoto’s patients tended to be high in the ratio.

Fig. 2 demonstrates a significant correlation between T4+/T8+ and mitogenic response of PMN to PHA in these patients and controls (r = 0.470.

**Table 1.**

Lymphocyte subpopulations of the patients with autoimmune thyroid diseases. Results are expressed as mean ± sem.

<table>
<thead>
<tr>
<th></th>
<th>No. of subjects</th>
<th>Lymphocyte count (µl)</th>
<th>OKT3+ (%)</th>
<th>OKT4+ (%)</th>
<th>OKT8+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22</td>
<td>1790 ± 400</td>
<td>71.1 ± 11.1</td>
<td>46.1 ± 9.5</td>
<td>34.5 ± 8.8</td>
</tr>
<tr>
<td>Graves’ disease</td>
<td>25</td>
<td>1820 ± 570</td>
<td>68.6 ± 10.7</td>
<td>50.6 ± 9.9</td>
<td>22.5 ± 7.6**</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>13</td>
<td>1870 ± 530</td>
<td>68.0 ± 10.0</td>
<td>49.0 ± 10.1</td>
<td>19.7 ± 6.1**</td>
</tr>
<tr>
<td>Euthyroid on drug</td>
<td>8</td>
<td>1950 ± 670</td>
<td>67.0 ± 11.7</td>
<td>47.7 ± 7.3</td>
<td>23.8 ± 8.1*</td>
</tr>
<tr>
<td>‘Euthyroid’ Graves’ disease</td>
<td>4</td>
<td>1390 ± 140</td>
<td>73.7 ± 10.9</td>
<td>61.2 ± 5.8</td>
<td>28.3 ± 6.5</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>16</td>
<td>1640 ± 470</td>
<td>69.0 ± 11.0</td>
<td>47.4 ± 12.0</td>
<td>29.5 ± 9.8</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>10</td>
<td>1810 ± 360</td>
<td>69.6 ± 8.3</td>
<td>48.8 ± 10.8</td>
<td>30.9 ± 11.6</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>6</td>
<td>1340 ± 480</td>
<td>68.2 ± 14.3</td>
<td>45.3 ± 13.5</td>
<td>27.3 ± 4.6</td>
</tr>
</tbody>
</table>

Significantly different from the value of control group: * P < 0.01; ** P < 0.001.
Fig. 1.

T4+/T8+ ratio of peripheral lymphocytes in autoimmune thyroid diseases. Bars represent mean ± SEM. Hyper.: hyperthyroid Graves' patients before treatment. 'Euthyroid' Graves' dis.: see Materials and Methods. On MMI: Graves' patients euthyroid on methimazole. Hypo. and Eu.: Hashimoto's patients in hypothyroid and euthyroid state, respectively, at the time of examination. * Significantly different from the value of control group ($P < 0.01$).

Fig. 2.

Correlation between T4+/T8+ ratio and PHA mitogenic response ($n = 31$, $r = 0.470$, $P < 0.01$). ● denotes Graves' patient; ▲ Hashimoto's patients; * control subject. All cases included in this figure are without medication.
Discussion

In the present study, a decrease of OKT8+ (suppressor/cytotoxic) T cells in the patients with Graves’ disease was noticed. Similar results have been reported employing the same panel of monoclonal antibodies by Thielemans et al. (1981) and Sridama et al. (1982), though we could not find significant difference of percentage of OKT3+ between patients and controls. The decrease of OKT8+ seems to be in accordance with the results of previous functional studies which suggested a decrease in the suppressor T lymphocyte function (Aoki et al. 1979; Okita et al. 1981). Calculation of the T4+/T8+ ratio made the relative diminution of OKT8+ subset more apparent in Graves’ patients and also suggested altered balance between the two subsets of T cells in hypothyroid Hashimoto’s patients, although to a lesser extent than in Graves’ patients.

Since thyroid status may influence the immune system, it seems important to determine whether or not these findings were secondary to the altered thyroid function. No correlation was observed between T4+/T8+ and serum levels of thyroid hormones. Further, ‘euthyroid’ Graves’ patients had a significantly reduced value of T4+/T8+. These findings indicate that the change of balance is rather primary in nature.

Unlike Sridama et al. (1982), we found a decrease of OKT8+ in Graves’ patients who were euthyroid on drug. The discrepancy may originate from the difference in extent of immunological remission, or from the difference of drugs used; methimazole for our patients and propylthiouracil for theirs.

It may not be surprising that T4+/T8+ showed positive correlation with PHA response, for the OKT4+ subset is known to be more strongly stimulated by this mitogen than the OKT8+ population (Reinherz & Schlossman 1980). However, like previous reports (Lundell et al. 1976; Mulaisho et al. 1975), we could not detect any significant difference in PHA response between patients and control subjects.

The decrease in the number of suppressor T lymphocytes might be followed by a development of forbidden clones of B cell lineage secreting autoantibodies. But no correlation was observed between T4+/T8+ and titres of thyroid autoantibodies. As for the relation of the ratio to the activity of TBII in Graves’ patients, our present data revealed no significant correlation. Thus, alteration in peripheral T cell subsets was not directly related to autoantibody levels in serum.

An imbalance of T cell subsets similar to that found in this study has also been reported in some other autoimmune diseases (Berrhi et al. 1981; Morimoto et al. 1980; Reinherz et al. 1980). Further studies on tissue-infiltrating lymphocytes with monoclonal antibodies, as were reported in multiple sclerosis (Nyland et al. 1982; Brinkman et al. 1982) and in rheumatoid arthritis (Janossy et al. 1981), will shed light on the pathogenetic significance of the alteration observed in peripheral T lymphocytes in these conditions.

References


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