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QUANTITATIVE AND FUNCTIONAL ASSESSMENT 
OF PERIPHERAL T-LYMPHOCYTES 
IN THYROID DISEASES 

By 
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ABSTRACT

A functional as well as quantitative assessment of the peripheral T-lymphocytes was made in 90 patients with various forms of thyroid diseases, 43 with Graves' disease (GD), 23 with non-toxic multinodular goitre (NTMG), 18 with toxic nodular goitre (TNG) and 12 with well-differentiated thyroid cancer (TC), using the E rosette-forming test and the local graft-versus-host reaction (GVHR). Forty healthy volunteers served as controls. In addition, the glands removed from the 38 patients who underwent operation were evaluated histologically for lymphocytic infiltration.

The percentage of E rosette-forming cells was found to be significantly lower than normal (58.0 ± 9.03) in the patients with GD (48.7 ± 9.83, \( P < 0.01 \)), NTMG (47.61 ± 12.54, \( P < 0.01 \)) and TNG (48.8 ± 11.1, \( P < 0.02 \)). The absolute T-cell count was significantly lower than normal (1207 ± 443) in the patients with GD (973 ± 331, \( P < 0.05 \)) and NTMG (890 ± 360, \( P < 0.02 \)). The GVHR was completely negative or weakly positive in only 2 of the 40 (5\%) controls, in 14 of the 39 (35.9\%) patients with GD, 14 of the 23 (60.8\%) with NTMG, 9 of the 17 (53\%) with TNG and 3 of the 12 (25\%) with TC. The amount of lymphocytic infiltration in the surgical specimen showed no correlation with the T-cell count and GVHR of the peripheral blood lymphocytes of the patients. Analysis of the data in the group as a whole also failed to reveal any correlation be-

 tween the GVHR and E rosette test. The GVHR is a functional test while the E rosette is a quantitative test. The findings of this study indicate that there is an impairment in the functional activity of the T-lymphocytes, patients with nodular goitre having shown a more pronounced impairment of cell-mediated immunity than those with GD and TC.

The role of a humoral autoimmune mechanism in thyroid diseases was first postulated some two decades ago (Roitt et al. 1956; Adams & Purves 1956; Blizzard et al. 1959). With the recent development of new laboratory techniques in this field, the participation of cell-mediated immunity (CMI) in thyroid diseases has also come under scrutiny. Sensitization towards thyroid antigens in some patients has been demonstrated by a number of methods, including skin testing (Buchanan et al. 1958), in vitro stimulation of the patient’s lymphocytes using lymphoblastic transformation (Delespesse et al. 1972) and tests on the inhibition of macrophage or leucocyte migration (Lamki et al. 1973; Delespesse et al. 1973).

It is the thymus-dependent lymphocytes, or T-cells, which are considered to be responsible for CMI phenomena. The quantitation of T-cells in thyroid disorders has, however, given widely varying results, from high levels (Aoki et al. 1973) to low levels (Hackenberg et al. 1975; Solda et al. 1975), with most investigators having reported normal levels (Wara et al. 1973; Urbaniak et al. 1974; Mulaiso et al. 1975; Volpé & Row 1975; Lundell et al. 1976).

In the presently-described study an assessment of the functional capacity of the peripheral T-lymphocytes of patients with thyroid disease has been made by means of the local xenogeneic graft-versus-host reaction in addition to the quantitative determination of their levels.

MATERIAL AND METHODS

The subject material comprised 90 patients with various forms of thyroid disease and 40 controls. These were divided into the following five groups: 1) Graves’ disease (GD), 43 patients, 33 females and 10 males aged from 18 to 75 years, all of whom had recently been diagnosed as being in the active stage of the disease and who had not yet received any treatment; 2) non-toxic multinodular goitre (NTMG), 23 patients, 22 females and 1 male aged from 21 to 58 years, 7 of whom had recently been diagnosed and the rest of whom had been under follow-up for various periods ranging from a few months to several years, with none receiving treatment; 3) toxic nodular goitre (TNG), 18 patients, 13 females and 5 males aged from 25 to 67 years, 14 of whom had recently been diagnosed and had never received any treatment; the other 4 who had received either propylthiouracil or deralin, but had never received any radioactive iodine, had been followed for a long time and had been without treatment for the past 6 months; 4) cancer of the thyroid (TC), 12 patients, 8 females and 4 males aged from 24 to 72 years, all of whom were tested prior to operation; in 6 cases the cancer was papillary, in 4 mixed
papillary-follicular (in 8 of the above 10 patients there was a metastatic spread to the regional lymph nodes), in one Hürthle cell and in one medullary cancer; 5) the 40 controls, 23 females and 17 males, aged from 16 to 67 years, were healthy volunteers with no signs of thyroid disease.

The diagnostic tests used included scanning of the thyroid, radioiodine uptake and, at the beginning of the study, determination of protein-bound iodine (PBI), which was later replaced by radioimmunoassay of T₄ and T₃. The patients with GD, 70% of whom had a diffuse goitre and 20% a visible exophthalmos, were all hyperactive, with no suppression as shown in the T₃ suppression test. In the patients with nodular goitre the clinically palpable finding was corroborated by scan and, in most of them, by the histopathological diagnosis of the operative specimen; the presence or absence of toxicity was determined on the appearance or lack of a “hot” nodule in the scan with suppressed extranodular tissue, hyperthyreotic clinical sign and symptoms, and a negative T₃ suppression test. The diagnosis of cancer was always based on histological examination of either a biopsy or operation specimen. It should be noted that evaluation of thyroid autoantibodies was made in only 4 patients and this factor was therefore not included in our assessment.

**Lymphocytic infiltration**

The glands removed in the 38 patients who underwent operation – 19 with colloid NTMG, 12 with TC, 4 with GD and 3 with TNG – were evaluated histologically for lymphocytic infiltration in an attempt to correlate the findings with those of the immunological tests. The classification of the degree of lymphocytic infiltration was made in accordance with the method of Vossmann & Seeliger (1969). 4 different sections of each gland being examined and scored from 0 to 4. It should be noted that all the thyrotoxic patients had been receiving treatment with propylthiouracil and iodine in preparation for the operation.

**Lymphocytes**

Lymphocytes were separated from the heparinized venous blood by the Ficoll-Hypaque sedimentation technique. At the same time a sequestrene blood sample was taken for a total and differential leukocyte count. The number of T-lymphocytes was determined according to the technique of spontaneous sheep red cell rosette formation, as described by Jondal et al. (1972). Correction of the results obtained was done by taking into consideration the number of monocytes contaminating the Ficoll-separated lymphocytes, which was determined by making a differential count of cytocentrifuged Giemsa-stained preparations obtained from the Ficoll-separated cell suspensions.

The functional activity of the lymphocytes was tested by means of a local xenogeneic graft-versus-host reaction (GVHR), as described by Shohat & Joshua (1976a), using a modification of the original method of Brent & Medawer (1966). A 0.1 ml sample of the lymphocyte suspension containing 20 x 10⁶ lymphocytes was injected intradermally into the closely-shaven abdominal skin of inbred Lewis rats weighing from 100 to 180 g which had been pre-treated 24 h earlier with 100 mg/kg cyclophosphamide (Cytoxan®). Five days later 0.4 ml of 1 % Evans blue was injected intravenously into each animal and 5 h later the entire abdominal skin was excised and the blue strain measured with calipers. A mean diameter of 2.0 mm or more was assessed as constituting a positive GVHR, from 1 to 2 mm as a weakly positive GVHR and less than 1 mm as a negative GVHR.
RESULTS

Table 1 presents the mean values of total lymphocytic counts as well as percentage and absolute number of E rosette-forming cells in the patient groups and the controls. The total lymphocyte counts showed no statistically significant differences from the normal. Significantly lower, however, were the mean relative and absolute values of E rosette in the GD and NTMG groups as well as the relative E rosette level in the TNG group. The group of TC patients showed no deviation from the normal levels of relative and absolute E rosettes.

Table 2 presents the results of the GVHR test. Whereas there was a negative or weakly positive GVHR in only 5% of the controls, 14 of the 39 GD patients (36%), 14 of the NTMG patients (61%), 9 of the 17 TNG patients (53%) and 3 of the 12 TC patients (25%) were unable to mount a positive GVHR. Thus there was significant deviation from the normal in all 4 groups of thyroid disease. It should be noted that when the mean diameter of the GVHR obtained in the TC group was compared with that of the controls the difference was not statistically significant; this may be due to the small number of patients in the TC group. Comparison of the mean diameters of the GVHR in the GD, NTMG and TNG groups with that in the controls showed a clear

<table>
<thead>
<tr>
<th>Group</th>
<th>Total lymphocyte count/mm³</th>
<th>E rosettes %</th>
<th>Absolute E rosette count/mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2068 ± 697</td>
<td>58 ± 9.03</td>
<td>1207 ± 443</td>
</tr>
<tr>
<td>GD</td>
<td>1975 ± 496</td>
<td>48.7 ± 0.83*</td>
<td>973 ± 331**</td>
</tr>
<tr>
<td>NTMG</td>
<td>1829 ± 545</td>
<td>47.61 ± 12.34*</td>
<td>890 ± 360**</td>
</tr>
<tr>
<td>TNG</td>
<td>2284 ± 839</td>
<td>48.8 ± 11.1**</td>
<td>1168 ± 657</td>
</tr>
<tr>
<td>TC</td>
<td>1856 ± 636</td>
<td>52.25 ± 11.98</td>
<td>967.75 ± 368</td>
</tr>
</tbody>
</table>

GD: Graves' disease. NTMG: non-toxic multinodular goitre. TNG: toxic nodular goitre. TC: thyroid cancer. Values marked by asterisks differ significantly from control by t-test: * P < 0.01; ** P < 0.02; *** P < 0.05.
Table 2.
Results of graft-versus-host reaction (GVHR) in various thyroid disorders.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of subjects</th>
<th>Positive GVHR (mm) mean ± sd</th>
<th>Diminished GVHR</th>
<th>Negative GVHR (mm) mean ± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40</td>
<td>38 (95 %)</td>
<td>--</td>
<td>2 (5 %)</td>
</tr>
<tr>
<td>GD</td>
<td>39</td>
<td>25 (64.1 %) +</td>
<td>4 (10.2 %)</td>
<td>10 (25.7 %)</td>
</tr>
<tr>
<td>NTMG</td>
<td>23</td>
<td>9 (39.1 %) +</td>
<td>3 (13.0 %)</td>
<td>11 (47.8 %)</td>
</tr>
<tr>
<td>TNG</td>
<td>17</td>
<td>8 (47 %) +</td>
<td>2 (11.7 %)</td>
<td>7 (41.3 %)</td>
</tr>
<tr>
<td>TC</td>
<td>12</td>
<td>9 (75 %) ++</td>
<td>--</td>
<td>3 (25 %)</td>
</tr>
</tbody>
</table>

GD: Graves' disease. NTMG: non-toxic multinodular goitre. TNG: toxic nodular goitre. TC: thyroid cancer. Values marked by crosses differ significantly from control by \( \chi^2 \) (Chi-square) test: + \( P < 0.001 \); ++ \( P < 0.02 \). Values marked by asterisk differ significantly from control by \( t \)-test: * \( P < 0.01 \).

Fig. 1.
T-lymphocyte (E rosette) percentage and graft-versus-host reaction (GVHR) in normal controls and patients with Graves' disease (GD), non-toxic multinodular goitre (NTMG), toxic nodular goitre (TNG) and thyroid cancer (TC).

56
Absolute T-cell count and graft-versus-host reaction (GVHR) in normal controls and patients with Graves' disease (GD), non-toxic multinodulad goitre (NTMG), toxic nodular goitre (TNG) and thyroid cancer (TC).

statistically-significant difference. Analysis of the individual results failed to show any correlation between the relative or absolute E rosette level and the GVHR; in all groups there were cases in which there was a negative GVHR although the E rosette count was normal (Figs. 1 and 2).

Table 3 presents the data obtained on the degree of lymphocytic infiltration found in the thyroid gland in the different disorders in relation to the percentage of peripheral T-cells, the absolute T-cell count and the GVHR. It is evident that no correlation could be established between these parameters.

**DISCUSSION**

The immunological reactions observed in different thyroid disorders, whether humoral or cell-mediated, have been various. Most of the reported studies were carried out in cases of GD and Hashimoto's thyroiditis. Early publications note the presence of high E rosette counts in both of these diseases (Aoki et al. 1973; Farid et al. 1973).
Urbaniak et al. (1974) found some non-consistent low levels of E rosettes in GD and in Hashimoto's thyroiditis but concluded from their data that T-cell levels are usually normal in these diseases. This was subsequently confirmed by the findings of Wara et al. (1973), Mulaisho et al. (1975) and Lundell et al. (1976) as well as by Volpé & Row (1975), who re-evaluated the results obtained previously reported by Farid et al. (1973). In contrast, the E rosette counts in patients with GD were reported by Hackenberg et al. (1975) to be slightly low and by Solda et al. (1975) to be markedly low. The latter investigators attributed this to the presence of lymphocytic infiltration in the thyroid gland. Folb & Bank (1976) found low E rosette counts in 4 of the 7 GD patients studied by them, and attributed these results to a variable distribution of T-lymphocytes in the peripheral blood.

Table 3.
Extent of lymphocytic infiltration in thyroid tissues of patients with various thyroid disorders, the relative and absolute peripheral T-cell counts and results of the GVHR.

<table>
<thead>
<tr>
<th>Score of lymphocytic infiltration</th>
<th>0</th>
<th>1*</th>
<th>2*</th>
<th>3*</th>
<th>4*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTMG</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>GD</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>TNG</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>12</td>
<td>12</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Mean</td>
<td>45.7</td>
<td>55.3</td>
<td>50.1</td>
<td>57</td>
<td>42.5</td>
</tr>
<tr>
<td>T %0</td>
<td>22-59</td>
<td>42-69</td>
<td>23-75</td>
<td>53-61</td>
<td>25-56</td>
</tr>
<tr>
<td>Mean Absolute T-count</td>
<td>1060</td>
<td>1234</td>
<td>830</td>
<td>1358</td>
<td>960</td>
</tr>
<tr>
<td>Negative and diminished over/total GVHR</td>
<td>3/7</td>
<td>5/10</td>
<td>4/11</td>
<td>1/2</td>
<td>1/5</td>
</tr>
</tbody>
</table>

0: No infiltrate.
1*: Minimal collection of lymphocytes.
2*: A few focal collections of lymphocytes with possible lymph follicle formation.
3*: Localized lymphocytic infiltration with a few lymph follicle formations.
4*: Diffuse collections of lymph follicles with rich lymphocytic infiltration.
There have been a number of reports on humoral immunity and CMI in cases of NTMG or diffuse goitre (Roitt et al. 1956; Senhauser et al. 1962; Saarma 1971; Delespesse et al. 1972, 1973). In a small group of patients with diffuse goitre the number of T-cells was found to be within normal limits (Hackenberg et al. 1975). The patients with this disorder in our study showed a significant decrease in both percentage and absolute number of T-cells. Of interest in relation to this point is the finding in our study of an evident lack of correlation between the lymphocytic infiltration present in the thyroid gland and the number of T-lymphocytes in the peripheral blood.

In the TNG group in our study the percentage of T-lymphocytes was significantly lower than normal, as in the NTMG and GD groups (Table 1), but the mean absolute T-cell count did not differ significantly from that of the control group due to the wide-spread of the absolute T-cell counts (Figs. 1 and 2). The absolute lymphocyte count as well as the absolute T-cell count were both somewhat higher in TNG than in the other thyroid disorders. It has been suggested that TNG has an aetiology and pathogenesis similar to that of NTMG, with the exception of differences in hormonal activity (Miller & Block 1970; Adams et al. 1975). It is possible that the higher level of hormonal activity in TNG causes a proliferation of non-T-cell elements, thus reducing the percentage of T-cells despite the increase in the total lymphocyte count.

Patients with slow-growing cancers of the thyroid have been found to have humoral and CMI reactions directed against either specific thyroid antigens or tumour antigens (Senhauser et al. 1962; Helmke & Federlin 1974; Amino et al. 1975; Rocklin et al. 1977). There has been a number of reports on the T-cell count in patients with solid tumours of organs other than the thyroid, the T-cell count usually having been found to be low when the cancer was wide-spread but normal or nearly normal when it was localized (Babusikova et al. 1975; Whitehead et al. 1976). In our patients with thyroid cancer, the percentage of T-lymphocytes was normal. The means of the total number of lymphocytes and absolute T-cell counts were slightly low, but this was statistically non-significant (Table 1).

This clear difference between the group of patients with TC and those with other forms of thyroid disease indicates the existence of different immunological mechanisms at work in these two groups.

The local GVHR, first introduced by Brent & Medawar (1966), constitutes a simple, accurate method for testing the functional integrity of human T-lymphocytes and has been used to assess CMI in cancer patients (Rees & Symes 1973; Shohat et al. 1974). Although it here reflects a xenogeneic reaction, this test has most of the characteristics of the allogeneic system or local GVHR studied in animal models (Rees & Symes 1973; Tatum & Lindquist 1974; Datta & Schwarz 1976). It requires competent donor T-cells although recipient B-cells are also necessary to elicit the reaction of the donor T-cells. Ford (1967) showed
that there is a direct relationship between the dose of lymphocytes injected and the magnitude of the local GVHR and a subsequent study in which two of the investigators participated (Shohat et al. 1976) demonstrated that the size of the xenogeneic local GVHR is proportional to the number of the lymphocytes injected so that the reaction can be quantitated. Shohat & Joshua (1976b) also found that use of an inoculum of $20 \times 10^6$ B-lymphocytes obtained from patients with chronic lymphocytic leukaemia gave no reaction. The GVHR is very sensitive and can be enhanced by a thymic humoral factor (THF), provided that precursor T-cells (the target cells) are present (Shohat et al. 1978b).

On the other hand, anti-T-lymphocyte serum can abolish it (Shohat et al. 1978a). In the present study, the percentage of negative and weakly positive GVHR was higher in the patients with NTMG (60.8%) and TNG (53%) than those with GD (35.9%) and TG (25%). We were unable to find any correlation between the GVHR and the metastatic phase of the tumour, possibly due to the slow growth of the thyroid cancer, and its metastases. The small number of patients in this group, however, does not allow us to draw any definite conclusions. The lack of correlation between the GVHR and the E rosette count (Figs. 1 and 2) is due to the different cellular properties upon which these tests are based. The E rosette-forming capacity of cells does not reflect the functional capacity of the T-cells, as has been documented both in the present and previous studies (Shohat et al. 1976; Shohat & Joshua 1976a). The involvement of additional factors in the pathogenesis of the various thyroid disorders may therefore be expressed as either a quantitative or functional impairment of the T-cells in individual patients.

In conclusion, it may be stated that the findings of the present study indicate the presence of a defective CMI in various thyroid diseases which was most pronounced in patients with nodular goitre and less so in patients with Graves' disease or thyroid cancer.

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REFERENCES


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