PROLIFERATION OF PERIPHERAL LYMPHOID CELLS
FROM EUTHYROID AND HYPERTHYROID INDIVIDUALS
CULTURED WITH HUMAN THYROGLOBULIN

By
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ABSTRACT
Peripheral lymphoid cells from euthyroid and hyperthyroid subjects were tested for proliferative responses to human thyroglobulin in vitro using [3H]thymidine incorporation as assay. Non-fractionated lymphoid cells from both groups displayed weak stimulation. Using a rosette technique for separating sub-populations of lymphoid cells it was concluded that T-cells constituted the responding cells. The results indicate that both hyperthyroid and euthyroid subjects possess clones of T-cells which are reactive against human thyroglobulin.

Thyroglobulin is a high molecular weight protein which is synthesized by thyroid epithelial cells and subsequently stored in the thyroid follicles. Under physiological conditions there is no leakage of this protein into surrounding tissues and lymphoid cells do not enter the follicles. In healthy subjects, as well as in patients with thyroiditis, there are B-lymphocytes which possess surface bound immunoglobulin receptors for thyroglobulin indicating that the immune system has the potential capacity to react against this substance (Urbaniak et al. 1973; Bankhurst et al. 1973). This concept is supported by the finding that experimental animals can readily be immunized against thyroglobulin resulting in the development of high titres of specific antibodies (Busci & Strausser 1972; Vladutiu & Rose 1975) and under certain conditions, when the thyroid parenchyma is known to be infiltrated by lymphocytes, there are frequently high anti-thyroglobulin titres (Roitt et al. 1956; Roitt & Doniach 1958; Lundell & Jonsson 1973). Moreover, peripheral lymphocytes from patients with thyroiditis exhibit marked DNA-synthetic responses to thyroglobulin in vitro (Ehren-
feld et al. 1971). Such a stimulation, although considerably weaker, has been recorded in hyperthyroidism (Ekrenfeld et al. 1971; Einhorn et al. 1971; Lundell et al. 1976).

In the present investigation we have examined whether the peripheral lymphoid cells of hyperthyroid individuals and healthy donors differ with regard to DNA-synthetic responses to thyroglobulin in vitro. We have also established whether the proliferating cell is a T- or a non-T-lymphocyte.

MATERIALS AND METHODS

Lymphocyte donors

The patient material consisted of 12 hyperthyroid females scheduled for 131I-therapy with a mean age of 61 years (range 47-74 years). The euthyroid donors consisted of 13 healthy volunteers, 11 females and 2 males, with no history of thyroid disease. These subjects had a mean age of 37 years (range 22 to 63 years).

Separation of lymphoid cells

The techniques for identifying and fractionating lymphoid cells have been described previously (Blomgren 1975). Briefly, lymphoid cells of heparinized venous blood were separated by centrifugation on Ficoll-Isopaque. These preparations of non-purified lymphocytes were washed twice by centrifugation in Eagle’s minimal essential medium supplemented with Earle’s salts (MEM). These suspensions were depleted of phagocytic cells by adding carbonyl iron powder followed by removal using a magnet. The remaining cells were incubated with sheep erythrocytes (SRBC) followed by centrifugation on Ficoll-Isopaque to fractionate T- and non-T-lymphocytes. The precipitating cells, e.g. those which had formed spontaneous rosettes with SRBC will be termed T-cell enriched and those which were collected from the fluid interphase will be termed B-cell enriched.

The frequency of T-cells in the preparations was determined by counting the proportion of cells forming rosettes with SRBC. The non-purified preparations contained 55-65% of T-cells and the T-cell enriched and B-cell enriched contained 90-95% and 6-10% respectively.

Thyroglobulin

One batch of human thyroglobulin was used. It was prepared from thyroid tissue removed during surgery according to a method described by others (Weibull & Linder 1960). It was dissolved in MEM and stored at -20°C before use.

Culture conditions

A microculture technique was employed which has been described previously (Lillie-höök & Blomgren 1974). Briefly, 2 x 10^5 lymphoid cells were cultured in the wells of microtest plates containing 0.2 ml of MEM supplemented with antibiotics and 10% of heat inactivated human serum from AB positive, Rh negative donors. Half of the cultures received thyroglobulin at a final concentration of 100 μg/ml and the others received no stimulants. After various periods of incubation at 37°C in a humified 5% CO2-air atmosphere each culture received 1.0 μCi of [3H]thymidine (specific
Table 1.

[\(^{3}\text{H}\)]thymidine incorporations, expressed as cpm, of various fractions of lymphoid cells from hyperthyroid and euthyroid individuals cultured with or without thyroglobulin. Mean values ± SE are presented.

<table>
<thead>
<tr>
<th>Cell donor</th>
<th>Lymphocyte fraction</th>
<th>Days of culture</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-purified</td>
<td>Thyroblulin Medium</td>
<td>1694 ± 328</td>
</tr>
<tr>
<td>B-cell enriched</td>
<td>Thyroblulin Medium</td>
<td>1648 ± 330</td>
</tr>
<tr>
<td>T-cell enriched</td>
<td>Thyroblulin Medium</td>
<td>3424 ± 845</td>
</tr>
<tr>
<td>Non-purified</td>
<td>Thyroblulin Medium</td>
<td>3225 ± 822</td>
</tr>
<tr>
<td>B-cell enriched</td>
<td>Thyroblulin Medium</td>
<td>854 ± 157</td>
</tr>
<tr>
<td>T-cell enriched</td>
<td>Thyroblulin Medium</td>
<td>708 ± 118</td>
</tr>
<tr>
<td>Euthyroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-purified</td>
<td>Thyroblulin Medium</td>
<td>1369 ± 272</td>
</tr>
<tr>
<td>B-cell enriched</td>
<td>Thyroblulin Medium</td>
<td>1013 ± 102</td>
</tr>
<tr>
<td>T-cell enriched</td>
<td>Thyroblulin Medium</td>
<td>2277 ± 231</td>
</tr>
<tr>
<td>Non-purified</td>
<td>Thyroblulin Medium</td>
<td>2343 ± 215</td>
</tr>
<tr>
<td>B-cell enriched</td>
<td>Thyroblulin Medium</td>
<td>1119 ± 136</td>
</tr>
<tr>
<td>T-cell enriched</td>
<td>Thyroblulin Medium</td>
<td>847 ± 102</td>
</tr>
</tbody>
</table>

* Numbers within brackets show the number of observations.
activity 5 Ci/mm. The Radiochemical Centre, Amersham, England). Twenty-four h later the cultures were terminated and incorporated radioactivity, expressed as counts per minute (cpm), determined as described previously (Lilliehöök & Blomgren 1974). Mean [3H]thymidine incorporation of 6–10 replicate determinations were calculated on an arithmetic basis.

Statistical analysis
To reduce the variability of the determinations, stimulation indices were calculated as the quotient between the stimulations obtained in cultures containing thyroglobulin and corresponding cultures without thyroglobulin. Indices higher than 1.0 thus indicate that thyroglobulin had stimulated cells to DNA-synthesis. The statistical method used was the Wilcoxon signed rank test.

RESULTS

Lymphoid cells from euthyroid and hyperthyroid donors were cultured with and without thyroglobulin. [3H]thymidine incorporations were determined on days 3, 6 and 9. Table 1 presents the mean isotope incorporations expressed as cpm. It can be seen that the three cell preparations, from both patients and

![Graph](image)

**Fig. 1.**

DNA-synthetic responses of various fractions of lymphoid cells cultured with thyroglobulin. Stimulations are expressed as indices (see Materials and Methods). The data are calculated from the results presented in Table 1. The left diagram shows the stimulation of T-cell enriched preparations, middle diagram B-cell enriched and right diagram non-purified cell preparations. Euthyroid donors ○—○, hyperthyroid donors ●—●. Asterisks at the symbols show the degree of statistical significance of the stimulation: *= P < 0.05, **= P < 0.01.
healthy donors, differed with respect to the extent of DNA-synthesis in the absence of thyroglobulin. This spontaneous activity was highest for the B-cell enriched preparations followed by the non-purified and T-cell enriched. Addition of thyroglobulin to the cultures enhanced [³H]thymidine incorporations of non-purified and T-cell enriched preparations. The differences, however, are not statistically significant.

In an attempt to minimize the variability, stimulation indices were calculated for each cell preparation. The mean values of these indices are depicted in Fig. 1. T-cell enriched preparations from euthyroid subjects exhibited significantly enhanced stimulations during the entire culture period. Nonwithstanding the large inter-test variability, it seems that T-cell enriched preparations from hyperthyroid subjects exhibit a smaller relative stimulation. On the 9th day of culture the [³H]thymidine incorporations of the euthyroid subjects' T-cells were significantly higher than those obtained from hyperthyroid donors ($P < 0.05$). B-cell enriched preparations from both euthyroid and hyperthyroid donors did not exhibit any significant stimulation by thyroglobulin; in contrast the spontaneous DNA-synthesis decreased somewhat when using cells from healthy donors. Non-purified preparations from both hyperthyroid and euthyroid subjects exhibited a tendency to become stimulated by thyroglobulin.

**DISCUSSION**

Patients with hyperthyroidism and thyroiditis frequently display high antibody titres against thyroglobulin and other thyroid constituents (Roitt et al. 1956; Roitt & Doniach 1958; Lundell & Jonsson 1973). The presence of such antibodies in hyperthyroid subjects before treatment has been shown to be positively linked to the development of hyperthyroidism following treatment with $^{131}I$ (Lundell & Jonsson 1973) or surgery (Hjort & Mogensen 1962). It is not definitely established whether these antibodies are of aetiological importance for the induction of hyperthyroidism or if they contribute to the development of hypothyroidism. Most subjects display the potential capacity to react immunologically against thyroglobulin. Specifically reactive B-lymphocytes can be identified in normal subjects by their binding of thyroglobulin to membrane associated immunoglobulins (Urbaniak et al. 1973; Bankhurst et al. 1973). However, specifically reactive T-cells cannot be detected by such a direct technique.

In this investigation we have tested whether peripheral lymphocytes from euthyroid and hyperthyroid subjects can be triggered to DNA-synthesis by human thyroglobulin in vitro, which would be an indication for the presence of specifically reactive lymphocytes. Such a tendency was noted using lymphocytes from both euthyroid and hyperthyroid donors and there was no detectable difference between the two groups which is in agreement with the results of other investigators (Ehrenfeld et al. 1971). Frationation of lymphocytes
established that T-cells, at least from euthyroid donors, were significantly stimulated by thyroglobulin. On the contrary, fractionated non-T-lymphocytes were not stimulated. Stimulation were overall very weak, which is to be expected when measuring a primary response of T-lymphocytes in vitro.

One explanation for our results could be that the DNA-synthetic response triggered by thyroglobulin is not due to antigen stimulation of the cells but rather due to a non-specific stimulatory effect of thyroid hormones (Lundell & Blomgren 1975) present on the thyroglobulin. However, we have measured the total contents of thyroxine and triiodothyronine in our cultures and found them to vary between 63–129 and 2.7–4.1 nmol/l, respectively. In previous experiments we have found that far higher concentrations of thyroid hormones are required to stimulate lymphocytes to DNA-synthesis in vitro (Lundell & Blomgren 1975).

In conclusion, our results indicate that T-cells are present in both euthyroid and hyperthyroid individuals, at approximately the same frequency, which are immunologically reactive against thyroglobulin. Since the stimulations were weak it is concluded either that the frequency of such T-cells is very low or that there are other regulatory cells which suppress their response.

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REFERENCES


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