*Clin. exp. Immunol.* (1983) **52,** 45–53.

# Thyroid autoantibody synthesis by cultures of thyroid and peripheral blood lymphocytes. I. Lymphocyte markers and response to pokeweed mitogen

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*(Accepted for publication 12 November 1982)*

## SUMMARY

Thyroid lymphocytes from Graves' and Hashimoto patients have been investigated and compared with lymphocytes from the peripheral blood. Considerably more lymphocytes ($20–30 \times 10^6$/g) could be isolated from Hashimoto thyroids than from Graves' tissue ($1–5 \times 10^6$/g) but the cell suspensions extracted from Hashimoto and Graves' glands were similar in terms of cell surface markers and the ability to synthesize immunoglobulin. Thyroid lymphocytes contained a lower proportion of T cells ($OKT3^+$ cells) and in some cases more B cells than the peripheral blood but the ratio of helper to suppressor T cells ($OKT4^+$:$OKT8^+$ cells) was similar to the values obtained for blood lymphocytes. Further, thyroid lymphocytes (unlike blood lymphocytes) synthesized relatively large amounts of microsomal and/or thyroglobulin antibody when cultured in medium only and these levels were significantly decreased by the addition of pokeweed mitogen. The results of this study provide further evidence for the role of the thyroid as a major site of thyroid autoantibody synthesis and emphasize the importance of characterizing the cells infiltrating the gland in autoimmune thyroid disease.

## INTRODUCTON

Antibodies to a number of thyroid antigens appear to be involved in the pathogenesis of autoimmune thyroid disease. In Hashimoto's thyroiditis, autoantibodies directed against thyroglobulin (Tg) and thyroid microsomes (Roitt *et al.*, 1956; Belyavin & Trotter, 1959) are associated with extensive lymphocytic infiltration of the gland and destruction of the thyroid epithelium (Biorklund & Soderstrom, 1976). In contrast, the hyperthryoidism of Graves' disease appears to be due to antibodies which bind to the TSH receptor and stimulate the thyroid by activating adenylate cyclase (reviewed by Rees Smith, 1981). The thyroid gland in Graves' patients is also infiltrated by lymphocytes (Swanson Beck *et al.*, 1973) and the presence of microsomal antibodies and less commonly Tg antibodies in such patients suggests a similarity in the pathogenesis of the autoimmune response in Hashimoto's and Graves' diseases. The mechanisms responsible for this breakdown in self-tolerance are unknown and consequently we have developed *in vitro* systems to enable us to investigate the synthesis of human thyroid autoantibodies (McLachlan *et al.*, 1977; McGregor *et al.*, 1979).

The source of lymphocytes most readily available for studies of thyroid autoantibody synthesis

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0009-9104/83/0400-0045$02.00 

is the peripheral blood but lymphocytes may also be isolated from thyroid tissue obtained at operation from patients with Graves' and Hashimoto's diseases. In this paper we report the results of our investigations on the yield of lymphocytes in relation to thyroid histopathology, T and B cell markers in lymphocyte suspensions from thyroid and peripheral blood and the ability of lymphocytes in these suspensions to synthesize immunoglobulins and thyroid autoantibodies in culture.

## MATERIALS AND METHODS

*Patients.* Graves' disease was diagnosed in two male and 10 female patients (mean age 36 years) on the basis of clinical features of hyperthyroidism as well as a diffuse thryoid scan, eye signs or the presence of thyroid microsomal antibodies (titres of 1:400 or greater as measured by TRC haemagglutination; Bird & Stephenson, 1973). Nine of these patients were treated with propranolol (80 mg tds) for 3–6 months prior to thyroidectomy; two patients received carbimazole initially followed by propranolol for 3–4 months and one patient was treated with carbimazole for 6 months (20 mg tds) before operation. Blood and thyroid tissue were obtained from these patients.

Thyroid tissue was also obtained from one male and three female Hashimoto patients (mean age 50 years). In addition, thyroid tissue became available as a result of investigative surgery for a thyroid nodule in two female patients (aged 63 and 64 years) whose sera were positive for thyroid autoantibodies. Peripheral blood from three of these patients and from a further 22 Hashimoto donors (18 female and four male, mean age 55 years) was obtained for culture purposes and from four of these and another eight Hashimoto patients (three male, nine female, mean age 58 years) for measurement of lymphocyte markers. All Hashimoto patients were euthyroid on thyroxine and had microsomal and/or Tg antibodies detectable by TRC haemagglutination (Bird & Stephenson, 1973; Cayzer, *et al*, 1978), the titres were $>1:10{,}000$ for Tg antibodies and $>1:1{,}600$ for microsomal (Mic) antibodies unless stated otherwise. Healthy laboratory and hospital staff (eight males and eight females, mean age 28 years) provided control donors for cell surface marker studies; lymphocytes from a second group (five male and nine female, mean age 40 years) were used for culture purposes.

*Thyroid histopathology.* Sections of thyroid tissue (5 $\mu$m, fixed in 10% formol saline and embedded in paraffin wax) were stained with haematoxylin & eosin in combination with methyl pyronin green to permit identification of plasma cells (Byers, 1977). 'Point-counting' of thyroid epithelial cells, lymphocytes and plasma cells was carried out on 10 random fields from thyroid tissue of each patient.

*Isolation and culture of lymphocyte suspensions.* Peripheral blood lymphocytes were isolated from heparinized venous blood (60–120 ml) by density gradient centrifugation (Böyum, 1976). Thyroid tissue (1–33 g) was teased apart in balanced salt solution to release mononuclear cells and the suspensions were purified on a Lymphoprep gradient. Similarly, lymphocytes were extracted from tonsil tissue (6 g) obtained at operation and from spleens (2–4g) available from kidney transplant donors. After extensive washing, cell viabilities were assessed by trypan blue exclusion (Boyse, Old & Chouroulinkov, 1964) and were >90% viable for peripheral blood lymphocytes and >80% viable for lymphocytes from thyroid tissue, spleen or tonsil. The lymphocytes were resuspended in culture medium (RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 $\mu$g/ml streptomycin and 0·25 $\mu$g/ml fungizone) and incubated at $8–10 \times 10^6$ lymphocytes/ml in 1 ml aliquots in Marbrook flasks (Marbrook, 1967) or at $0{\cdot}5 \times 10^6$/ml in 1 ml aliquots in round bottomed tubes (Falcon 2054). Cultures were set up (three or four replicates) with or without pokeweed mitogen (PWM, GIBCO, New York, USA; 3 $\mu$l/ml). In addition, thyroid lymphocytes from one patient were cultured after infection with Epstein–Barr virus (EBV) using supernatants from the B95–8 cell line (Bird & Britton, 1979) in the presence of cyclosporin A (Sandoz, 0·2 $\mu$g/ml). After a culture interval of 14 days at 37°C in an atmosphere of 5% carbon dioxide in air, cultures were harvested by centrifugation at 400*g* and the supernatants stored at −70°C.

*T and B cell markers.* Cell suspensions were assayed for B cells by indirect immunofluorescence

of surface immunoglobulin (Brown & Greaves, 1974) using rabbit anti-human immunoglobulin and fluorescein conjugated goat anti-rabbit immunoglobulin (Wellcome Laboratories). The numbers of total T cells ($OKT3^+$) and T cell subsets ($OKT4^+$ and $OKT8^+$) were determined by indirect immunofluorescence using monoclonal antisera and goat anti-mouse immunoglobulin conjugated to fluorescein (Ortho Pharmaceuticals). Cell suspensions were shown by differential staining to contain more than 75% lymphocytes (mean ± s.e. (mean), 87 ± 2%, $n = 17$ for blood lymphocytes and 90 ± 5%, $n = 5$ for thyroid lymphocytes) and less than 20% monocytes (13 ± 2% for blood lymphocytes and 10 ± 5% for thyroid lymphocytes). The contribution made to cell suspensions by monocytes was taken into consideration when evaluating T and B cell markers and the results have, therefore, been expressed as a percentage of the total number of lymphocytes.

*Analysis of immunoglobulins synthesized in culture.* Total IgG was measured directly in culture supernatants using a solid phase radioimmunoassay (McLachlan, Rees Smith & Hall, 1978). Thyroid autoantibodies were determined by TRC haemagglutination or by ELISA. The ELISA techniques used to measure Tg and Mic antibodies synthesized in culture have been described in detail elsewhere (McLachlan *et al.*, 1982; Schardt *et al.*, 1982). Aliquots (100 μl) of culture supernatant were assayed in duplicate and the results have been expressed as an ELISA Index as follows:

$$\text{ELISA index} = \frac{\text{Optical density of test sample}}{\text{Optical density of a standard serum dilution}}.$$

When Tg antibodies were being measured, the standard was a sample of Hashimoto serum (haemagglutination titre 1:5,120) diluted 400 times; in Mic antibody ELISA measurements, the reference serum had a haemagglutination titre of 1:204,800 and was diluted 4,000 times. The significance of differences between background values (culture medium alone) and culture supernatants (or between culture superntants from the same lymphocytes studied under different conditions) was tested by Student's *t*-test.

## RESULTS

*Yield of thyroid lymphocytes in relation to the histopatholgy of the gland*

In sections of Hashimoto glands, lymphoid cells occurred more than three times as frequently as epithelial cells whereas in Graves' tissue epithelial cells were 3–15 times as common as lymphocytes in glands showing features of focal thyroiditis and over 50 times more frequent in purely thyrotoxic tissue (Table 1). The yield of lymphocytes was correlated with the extent of lymphocytic infiltration, being greatest from Hashimoto thyroids and least from thyrotoxic glands. The proportion of lymphoid to plasma cells was similar in Hashimoto glands and Graves' tissue with associated thyroiditis but plasma cells and germinal centres were absent from thyrotoxic tissue (Table 1).

**Table 1.** Thyroid histopathology and yield of lymphocytes from thyroid tissue in patients with Graves' and Hashimoto's diseases. The number of individuals studied is given in parentheses

| | | Graves' disease | |
|---|---|---|---|
| | Hashimoto's disease (4) | With thyroiditis (7) | Without thyroiditis (4) |
| Lymphoid: epithelial cells (range) | 7:1–3:1 | 1:3–1:15 | 1:55–1:130 |
| Lymphoid: plasma cells (range) | 18:1–15:1 | 19:1–3:1 | Plasma cells absent |
| Germinal centres | Present | Present | Absent |
| Yield of lymphocytes $\times 10^6$/g (mean ± s.e. (mean)) | 22·5 ± 3·9 | 6·5 ± 3·9 | 1·6 ± 1·1 |

*Lymphocyte markers in thyroid and peripheral blood cell suspensions*

T and B cell surface markers were studied in thyroid lymphocytes from seven propranolol treated Graves' patients and one Hashimoto patient as well as in peripheral blood lymphocytes obtained preoperatively from these seven individuals with Graves' disease. Lymphocyte markers were also investigated in a panel of 13 Hashimoto patients (Mic and Tg antibody titres $>1:1{,}600$ and $>1:10{,}000$ respectively) and a panel of 13 normal donors. The percentage of B cells was similar in peripheral blood lymphocytes from Hashimoto and normal donors but blood lymphocytes from the Graves' patients contained a higher proportion of B cells than either Hashimoto or normal peripheral blood lymphocytes ($P<0{\cdot}05$, Table 2). Graves' thyroid lymphocyte suspensions contained from 3–34% B cells and although there was a tendency for the proportion of B cells to be higher in thyroid lymphocyte suspensions than in the blood, the difference was not significant.

Analysis of T cells using monoclonal antisera demonstrated that there was no significant difference in the percentage of $OKT3^+$ or $OKT8^+$ cells in the peripheral blood of Hashimoto, Graves' or normal individuals and although the proportion of $OKT4^+$ cells was higher in Graves' and Hashimoto patients than in normal donors ($P<0{\cdot}05$), the ratio of $OKT4^+$:$OKT8^+$ cells was similar in blood lymphocytes from the three groups of individuals (Table 2). In contrast, thyroid lymphocytes from the seven Graves' patients contained significantly fewer total T cells ($OKT3^+$) and $OKT4^+$ cells than peripheral blood ($P<0{\cdot}02$ and $<0{\cdot}002$ respectively) although the proportion of $OKT8^+$ cells and the ratio of $OKT4^+$:$OKT8^+$ cells did not differ from the levels in the blood lymphocytes ($P>0{\cdot}05$, Table 2).

Lymphocytes isolated from thyroid tissue of Hashimoto patient MS were thawed after storage in 10% dimethyl sulphoxide at $-196$°C and analysed for cell markers. The values obtained for % B cells, total T cells and T cells subsets as well as the $OKT4^+$:$OKT8^+$ ratio fell within the range observed for thyroid lymphocytes from the Graves' patients studied (Table 2).

Thyroid lymphocytes were also studied in two patients undergoing surgery for a thyroid nodule: cell suspensions were prepared from tissue distal to the suspected nodule and in patient DC the % B lymphocytes and the $OKT4^+$:OKT8 ratio were similar to the values obtained for lymphocytes from Graves' and Hashimoto glands (Table 2). In contrast, thyroid lymphocytes from patient EL contained a high proportion of $OKT4^+$ cells and a low proportion of $OKT8^+$ cells and consequently the $OKT4^+$:$OKT8^+$ ratio was markedly greater than the value obtained for any other individual studied (Table 2). The inoperable papillary carcinoma in this patient was

**Table 2.** Proportion of T cells and B cells present in lymphocytes from the peripheral blood and thyroid tissue of patients with autoimmune thyroid disease; data are included for a panel of 12 normal individuals. Results are given as the mean (±s.e. (mean)) for positive cells expressed as a % of the total number of lymphocytes; the number of individuals studied is given in parentheses

| | Peripheral blood lymphocytes | | | Thyroid lymphocytes | | | |
|---|---|---|---|---|---|---|---|
| Cell marker | Graves' (6) | Hashimoto (13) | Normal (12) | Graves' (7) | Hashimoto MS* | Thyroid nodule EL | DC |
| *T cells* | | | | | | | |
| % $OKT3^+$ cells | 79±5 | 81±2 | 82±2 | 62±5‡ | 59 | 76 | 87 |
| % $OKT4^+$ cells | 58±6† | 57±4† | 50±2 | 39±4§ | 40 | 64 | 54 |
| % $OKT8^+$ cells | 28±9 | 30±2 | 32±2 | 31±4 | 22 | 11 | 45 |
| $OKT4^+$:$OKT8^+$ ratio | 2·3±0·9 | 2·0±0·2 | 1·7±0·2 | 1·4±0·2 | 1·8 | 5·8 | 1·2 |
| *B cells* | | | | | | | |
| % Surface immunoglobulin$^+$ cells | 16±4† | 8±2 | 7±1 | 19±5 | 28 | 30 | 20 |

*Results obtained from lymphocytes stored at $-196$°C

Values significantly different from Graves' peripheral blood lymphocytes: ‡ $P<0{\cdot}02$; §$P<0{\cdot}002$.

Values significantly different from normal peripheral blood lymphocytes: †$P<0{\cdot}05$.

subsequently found to be extensively infiltrated by lymphocytes and it is possible that the lymphocytes extracted from the biopsy specimen included cells associated with the tumour as well as those involved in thyroid damage.

*Total IgG synthesis by thyroid and peripheral blood lymphocytes*

Thyroid lymphocytes from three Hashimoto and four Graves' patients synthesized as much total IgG when cultured in medium only as in cultures to which PWM was added and similar results were obtained for lymphocytes from thyroid tissue of patient DC who had a thyroid nodule (Table 3). In

**Table 3.** IgG synthesis by thyroid and peripheral blood lymphocytes from Graves' and Hashimoto patients cultured for 14 days in the presence or absence of PWM. Data are given as mean ± s.e. (mean) and values are included for IgG synthesis by lymphocytes from the blood, spleen and tonsil of normal individuals. The number of replicate cultures is given by *n*

| Clinical status | Donor | *n* | μg IgG synthesized/$10^7$ cells | | Ratio |
|---|---|---|---|---|---|
| | | | −PWM | +PWM | +PWM:−PWM |
| *Thyroid lymphocytes* | | | | | |
| Hashimoto | DJ | 4 | 247·6 ± 31·4 | 206·3 ± 44·0 | 0·8 |
| Patients | JH | 5 | 134·9 ± 19·3 | 89·5 ± 4·7 | 0·7 |
| | MS | 4 | 11·1 ± 2·3 | 9·0 ± 1·0 | 0·8 |
| Thyroid | DC* | 3 | 3·1 ± 0·4 | 3·4 ± 0·1 | 1·1 |
| Nodule | EL | 4 | 1·2 ± 0·3 | 22·9 ± 4·4‡ | 19·1 |
| Graves' | MA | 4 | 7·1 ± 1·6 | 11·1 ± 1·7 | 1·6 |
| Patients | JL* | 4 | 36·9 ± 2·8† | 7·2 ± 1·1 | 0·2 |
| | BG* | 4 | 18·1–1·7† | 3·4 ± 0·4 | 0·2 |
| | SE | 2 | ND | 17·5 | — |
| | DW | 4 | ND | 15·2 ± 0·2 | — |
| | WS | 2 | ND | 5·8 | — |
| | IJ | 2–3 | 0·9 ± 0·1 | 0·6 | 0·7 |
| *Peripheral blood lymphocytes* | | | | | |
| Hashimoto Patient | MS | 3 | 3·7 ± 0·9 | 14·4 ± 1·7‡ | 3·9 |
| Thyroid | DC* | 4 | 1·1 ± 0·3 | 54·5 ± 4·1§ | 49·5 |
| Nodule | EL | 3 | 1·1 ± 0·3 | 12·1 ± 1·2§ | 11·0 |
| Graves' | MA | 4 | ND | 73·2 ± 9·7 | — |
| Patients | JL | 3 | 1·3 ± 0·1 | 31·3 ± 3·6§ | 24·1 |
| | BG | 4 | 3·5 ± 0·6 | 62·7 ± 18·7‡ | 17·9 |
| | IJ | 4 | 3·8 ± 1·2 | 94·8 ± 29·2‡ | 25·0 |
| Hashimoto | Panel of individuals | 22 | 3·3 ± 0·8 | 131·8 ± 36·4§ | 56·4 ± 13·2 |
| Normal | Panel of individuals | 14 | 3·8 ± 1·5 | 108·0 ± 16·8§ | 53·5 ± 23·3 |
| *Spleen lymphocytes* | | | | | |
| Normal | I | 3 | 4·8 ± 1·1 | 66·8 ± 30·9 | 13·9 |
| | II | 4 | 23·2 ± 5·1 | 1226·1 ± 119·0§ | 53·0 |
| | III | 4 | 5·4 ± 1·1 | 127·1 ± 17·3§ | 23·5 |
| *Tonsil lymphocytes* | | | | | |
| Normal | IV | 2–3 | 32·8 | 74·7 ± 7·45‡ | 2·3 |

ND = Not determined; *Lymphocytes cultured in tubes.

Values significantly lower than those obtained for lymphocytes cultured with PWM †$P < 0{\cdot}001$

Values significantly greater than those obtained for lymphocytes cultured in medium only: ‡$P < 0{\cdot}05$; §$P < 0{\cdot}001$

two of these cultures (patients JL and BG) the presence of PWM was associated with a significant decrease in IgG production. Lymphocytes from tissue of patient EL (who had a thyroid carcinoma) synthesized more IgG in the presence of PWM than in its absence (Table 3). With this exception, IgG synthesis by thyroid lymphocytes was not enhanced by the addition of the mitogen.

In contrast, peripheral blood lymphocytes from six of these patients produced 3–50 times as much IgG when incubated with PWM and these results are in agreement with those obtained from a panel of 22 Hashimoto patients and 14 control donors (Table 3). It seems unlikely that the culture conditions were only favourable for peripheral blood lymphocytes since lymphocytes extracted from a tonsil and from spleen tissue of two out of three donors responded significantly to the same dose of PWM (Table 3). It is possible to stimulate thyroid lymphocytes using Epstein–Barr virus as an activator: virally infected lymphocytes from the thyroid of patient MS synthesized $67{\cdot}0 \pm 4{\cdot}9$ ($n=9$) $\mu$g IgG/$10^7$ lymphocytes compared with $11{\cdot}1 \pm 2{\cdot}3$ $\mu$g/$10^7$ cells in the absence of virus or mitogen.

The amounts of IgG synthesized by thyroid lymphocytes varied in different patients, the highest values being observed in cultures of lymphocytes from tissue in which germinal centres were common (Hashimoto patients DJ and JH, Table 3). The lowest level of IgG production was recorded in cultures of lymphocytes isolated from thyrotoxic tissue of patient IJ who had been treated with carbimazole throughout the pre-operative period (Table 3). The IgG secreted by thyroid lymphocytes was the product of active secretion as evidenced by kinetic studies with lymphocytes from patient MA which demonstrated increasing amounts of IgG as follows: $1{\cdot}8 \pm 0{\cdot}1$ at 3 days, $4{\cdot}7 \pm 0{\cdot}7$ at 7 days and $7{\cdot}1 \pm 1{\cdot}6$ $\mu$g/$10^7$ cells after 14 days.

**Table 4.** Synthesis of thyroid autoantibodies (measured by haemagglutination or ELISA) in cultures of thyroid or peripheral blood lymphocytes from patients with autoimmune thyroid disease. Results are expressed as the mean ± s.e. (mean) (where $n$ is the number of replicate cultures).

| | | | Thyroglobulin antibody | | Microsomal antibody | |
|---|---|---|---|---|---|---|
| Patient | Donor | $n$ | −PWM | +PWM | −PWM | +PWM |
| *Thyroid lymphocytes: haemagglutination (reciprocal titre)* | | | | | | |
| Hashimoto | JH | 5 | 168±45 | 96±27 | 336±89 | 104±24† |
| Patients | DJ | 4 | 40±0 | 15±3‡ | Undetectable | Undetectable |
| *Thyroid lymphocytes: ELISA Index* | | | | | | |
| Hashimoto | | | | | | |
| Patient | MS | 4 | 0·14±0·02 | 0·07±0·01‡ | 1·37±0·03 | 1·05±0·02† |
| Thyroid | DC* | 3 | 0·37±0·01 | 0·38±0·02 | Undetectable | Undetectable |
| Nodule | EL* | 4 | Undetectable | Undetectable | Undetectable | Undetectable |
| Graves' | MA | 4 | 0·24±0·06 | 0·17±0·03 | 0·06±0·01 | Undetectable† |
| Patients | JL* | 4 | 0·36±0·05 | 0·09±0·02‡ | 0·57±0·07 | 0·11±0·02† |
| | BG* | 4 | Undetectable | Undetectable | 0·05±0·01 | Undetectable† |
| *Peripheral blood lymphocytes: ELISA Index* | | | | | | |
| Hashimoto | MS | 3 | Undetectable | Undetectable | 0·45±0·07 | 1·17±0·10§ |
| Thyroid | DC | 3 | 0·14±0·02 | 0·22±0·04 | Undetectable | Undetectable |
| Nodule | EL | 3 | Undetectable | Undetectable | Undetectable | Undetectable |
| Graves' | MA | 4 | ND | 0·06±0·01 | ND | 0·07±0·02 |
| Patients | JL | 4 | Undetectable | 0·13±0·03§ | Undetectable | 0·44±0·10§ |
| | BG | 4 | Undetectable | Undetectable | Undetectable | 0·14±0·09§ |

Values significantly lower than those obtained for lymphocytes cultured in medium only: †$P<0{\cdot}05$; ‡$P<0{\cdot}001$.

Values significantly greater than those obtained for lymphocytes cultured in medium only: §$P<0{\cdot}05$.

*Lymphocytes cultured in tubes. ND = not determined.

*Synthesis of thyroid autoantibodies*

Thyroid autoantibodies (measured by haemagglutination or ELISA) were detectable in cultures of thyroid lymphocytes from the three Hashimoto and three Graves' patients studied (Table 4). Lymphocytes from patients JH, MS, MA and JL (whose sera were positive for Mic and Tg antibodies) synthesized both Mic and Tg antibodies *in vitro* whereas cells isolated from thyroid tissue of patients DJ, MS and BG produced Mic or Tg antibodies in accordance with the type of thyroid autoantibody present in serum. In the presence of PWM, the amount of thyroid autoantibody synthesized was decreased, usually significantly ($P < 0{\cdot}05$, Table 4).

The results obtained using tissue from patients with suspected thyroid nodules were different: lymphocytes extracted from the thyroid of patient EL did not synthesize detectable levels of thyroid autoantibodies and although thyroid lymphocytes from patient DC produced measurable amounts of Tg antibody when cultured in medium only, the addition of PWM had no effect on autoantibody synthesis (Table 4). Although thyroid tissue from thyroid autoantibody positive patients undergoing investigative surgery for a thyroid nodule is potentially of considerable use in furthering our understanding of autoimmune thyroid disease, it is likely that lymphocytes from these glands may not be characteristic of the cells associated with the lesions in Hashimoto's and Graves' diseases.

Autoantibody synthesis was also monitored in cultures of peripheral blood lymphocytes from six of the eight patients studied. Mic antibodies were synthesized in unstimulated cultures of peripheral blood lymphocytes from patient MS (who had a serum Mic antibody titre of 1:204,800) and the level of Mic antibody production was increased in cultures to which PWM had been added (Table 4). Similarly, peripheral blood lymphocytes from patient DC, (with a Tg antibody titre of 1:10,000) produced Tg antibody in the absence of PWM and these levels were also slightly increased (but not significantly) in PWM stimulated cultures. Cultures of peripheral blood lymphocytes from three Graves' patients with lower serum levels of thyroid autoantibodies (less than 1:800 for Mic or Tg antibody) only synthesized detectable amounts of thyroid autoantibodies when PWM was present in culture (Table 4).

## DISCUSSION

Lymphocytes isolated from thyroid tissue of Graves' and Hashimoto patients have been characterized in terms of cell surface markers and immunoglobulin synthesis and compared with peripheral blood lymphocytes. The yield of lymphocytes was greatest from Hashimoto glands, intermediate in Graves' glands with associated thyroiditis and least in purely thyrotoxic tissue and these results were in agreement with the extent of lymphocytic infiltration observed in sections of thyroid tissue.

Lymphocyte suspensions extracted from thyroid tissue of seven Graves' patients and one Hashimoto patient contained fewer total T cells ($OKT3^+$) and T cells of the subset $OKT4^+$ than lymphocytes in the peripheral blood. Our observations of an increase in the percentage of B cells in Hashimoto and some Graves' thyroid lymphocytes and a reduction in T cells in Graves' thyroid lymphocytes compared with the peripheral blood are in agreement with the results obtained by Totterman (1978) for thyroid aspirates. In general, no significant difference was observed between peripheral blood and thyroid lymphocytes in terms of the ratio of helper: suppressor T cell subsets as defined by the monoclonal antisera OKT4 and OKT8 (Reinherz *et al.*, 1980). However, it has been shown that the $OKT4^+$ subset is heterogenous and includes cells capable of suppressing immunoglobulin production (Thomas *et al.*, 1981). Perhaps the most important difference observed between thyroid and blood lymphocytes was the increase in B lymphocytes relative to T cells as it is likely that this could radically alter the activity of thyroid derived B lymphocytes *in vitro* and *in vivo*.

The presence of plasma cells and germinal centres observed in thyroid sections was associated with the ability of lymphocytes isolated from these tissues to synthesize IgG and thyroid autoantibody in culture in the absence of mitogenic triggering; these data are in agreement with previous studies demonstrating thyroid autoantibody production by thyroid lymphocytes from Hashimoto and Graves' patients (McLachlan, *et al.*, 1979; Weetman *et al.*, 1982). The present

studies have shown that thyroid lymphocytes were not stimulated by PWM to produce increased amounts of IgG under conditions which stimulated Hashimoto and normal peripheral blood lymphocytes or spleen lymphocytes from normal donors. Further, the addition of PWM inhibited the synthesis of thyroid autoantibody by thyroid lymphocytes. Similar observations were made by Stevens *et al.* (1979) in studies of tetanus toxoid antibody synthesis by peripheral blood lymphocytes: 10–14 days after boosting with the toxoid, lymphoblastoid cells are circulating which spontaneously secrete antibody to tetanus toxoid and this secretion is inhibited by the action of PWM.

The difference between thyroid and peripheral blood lymphocytes in terms of T and B cells, the ability to secrete thyroid autoantibodies and the response to PWM suggested that the influence of antigen might also differ in lymphocytes from these two types of tissue. The results of our studies using Tg to stimulate Tg antibody synthesis by thyroid and peripheral blood lymphocytes are reported in a subsequent paper.

We are most grateful to Mrs Patricia Rooke, Mrs Marian Atherton, Miss Sharron George and Mr B. Phail for technical assistance and Mrs C. Jack for secretarial assistance. We would like to thank Professor I. Johnston, Mr L.B. Fleming and Mr P.D. Hindmarsh, Royal Victoria Infirmary and Mr G. Proud, Freeman Hospital for providing us with clinical material and Dr T. Bell, Royal Victoria Infirmary for B95-8 supernatants. The work was supported by grants from: The Medical Research Council, The Wellcome Trust, the North of England Campaign for Cancer Research, the League of Friends of the Royal Victoria Infirmary, the Clement Lawson Fund and the Scientific and Research Committee of the Newcastle Area Health Authority.

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