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Heterogeneity of immunoregulatory T cells in human thyroid autoimmunity: influence of thyroid status

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SUMMARY

Monoclonal antibodies of the OKT series were used to identify circulating T lymphocytes ($OKT3^+$), their helper–inducer ($OKT4^+$) and suppressor–cytotoxic ($OKT8^+$) subsets and cells bearing Ia antigen ($OKIa^+$) in 75 patients with thyroid autoimmune disorders, including 14 Graves' disease, 21 myxoedema, 20 asymptomatic thyroiditis, 12 Hashimoto's thyroiditis and eight simple goitre with superimposed thyroiditis. In the whole population of patients, a negative correlation was observed between the percentage of $OKT8^+$ cells and serum free thyroxine levels whatever the type of thyroiditis. The percentage of $OKT8^+$ cells was decreased in Graves' disease and increased in myxoedema while it reversed after adequate treatment of the two diseases. However, a trend to a decrease in the proportion of $OKT8^+$ cells was still observed in treated Graves' disease and in all the other groups of thyroiditis with euthyroidism. The minor modifications observed for $OKT3^+$ and $OKT4^+$ cells were in relation with those of $OKT8^+$ cells. There was an increased percentage of Ia^+ cells in Graves' disease and in Hashimoto's thyroiditis partly reflecting the presence of activated lymphocytes. In conclusion, these data suggest first of all a direct influence of serum T4 on the distribution of circulating $OKT8^+$ cells in addition to documenting the heterogeneity of T cell immunoregulatory factors.

INTRODUCTION

Graves' disease, Hashimoto's goitre and asymptomatic thyroiditis with or without spontaneous myxoedema, are currently considered to be organ specific autoimmune diseases (Bastenie & Ermans, 1972). Recently, evidence for a decrease in suppressor T lymphocyte function (Aoki, Pinnamaneni & DeGroot, 1979; Okita, Row & Volpe, 1981) and a reduction in the percentage of suppressor–cytotoxic cells (Thielemans *et al.*, 1981; Sridama, Pacini & DeGroot, 1982) have been considered as documenting a deficit in suppressor activity (Thielemans *et al.*, 1981) in various types of autoimmune thyroiditis. However, the thyroid status *per se*, hyper- and hypothyroidism, may influence the performance of the immune system (Fabris, 1973; Bonnyns *et al.*, 1978; Basso, Mocchegiani & Fabris, 1981). Moreover, low levels of suppressor cells can be related to decreased production or to enhanced trapping (de Sousa, 1981). Therefore, using monoclonal antibodies of the OKT series, T lymphocyte subsets and circulating activated lymphocytes were reassessed in thyroid autoimmunity in relation with the type of thyroiditis and the thyroid function.

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MATERIALS AND METHODS

Subjects. Seventy-five patients with thyroid autoimmune disorders were studied. They comprised 67 females and eight males aged between 16 and 82 years (mean 46 years). On the basis of classic clinical and biological criteria, the patients were diagnosed as Graves' disease (14), myxoedema (21), asymptomatic thyroiditis (20), Hashimoto's goitre (12) and simple goitre with low levels of thyroid antibodies (eight). Each group was subdivided depending on the treatment or its absence (Table 1). All the treated patients were euthyroid. In Graves' disease, they had received methimazole, ^{131}I or had been submitted to subtotal thyroidectomy; the other treated patients were on substitution therapy with L-thyroxine. Twenty-one healthy adults matched for age and sex were used to establish the normal control values.

Thyroid tests. Serum total thyroid hormones (T4 and T3), thyroxine binding globulin (TBG) and thyrotropic hormone (TSH) were measured by classic radioimmunoassays. The free T4 index (FT4 I) was calculated from T4 and TBG values (normal range: 0·80–2·10 ng/dl).

Thyroid antibodies. Thyroglobulin antibodies (TGA) were detected by a radioimmunological method (Delespesse *et al.*, 1976) and thyroid microsomal antibodies with an immunofluorescent technique.

Lymphocyte markers. Monoclonal antibodies were obtained from Ortho Pharmaceutical Corporation (Raritan, New Jersey, USA). The antibodies used in this study were directed against mature thymocytes and all peripheral blood T cells ($OKT3^+$), T helper cells ($OKT4^+$), T suppressor–cytotoxic cells ($OKT8^+$), Ia antigen ($OKIa^+$). The specificity of these antibodies has been documented (Reinherz *et al.*, 1979a, 1979b, 1980). The preparation of fluorescent peripheral blood mononuclear cells has been previously described (Mascart-Lemone *et al.*, 1982). In the beginning of this study, fluorescent cells were counted on a Leitz fluorescent microscope (Mascart-Lemone *et al.*, 1982). Later, the fluorescent cells were analysed by a flow cytofluorometry technique which allows to distinguish lymphocytes from other leucocytes on the basis of their light scattering properties (Hoffman *et al.*, 1980). The latter technique improved the accuracy of the results. In order to use the data obtained by both manual and automated techniques of counting, results were expressed in percentage of the control means, defined as 100%.

Statistical analysis. This was performed by the Student's *t*-test. Linear regression was calculated by the least square method.

RESULTS

As shown in Table 1, statistically significant differences in T cells subsets were found in the following groups of patients with respect to normal controls: (1) $OKT3^+$ cells were lower in Graves' disease (toxic and euthyroid), in treated myxoedema, in treated Hashimoto's goitre and in asymptomatic thyroiditis without medication; (2) $OKT4^+$ cells were decreased in untreated Graves' disease; (3) $OKT8^+$ cells were reduced in toxic Graves' disease, in treated myxoedema and in patients with asymptomatic thyroiditis on thyroxine; (4) $OKIa^+$ cells were increased in toxic Graves' disease and in treated Hashimoto's goitre; (5) the $OKT4^+$ to $OKT8^+$ ratio was increased in treated myxoedema. The percentages of T cell subsets were also significantly different in untreated Graves' disease ($OKT8^+$ and $OKIa^+$) and in myxoedema ($OKT3^+$, $OKT8^+$ and the $OKT4^+$ to $OKT8^+$ ratio) compared to those found in the corresponding euthyroid group. No difference was found in T cells subsets between untreated and thyroxine treated patients with asymptomatic thyroiditis, Hashimoto's thyroiditis and simple goitre with thyroiditis. When untreated and treated patients were grouped, there was a significant decrease of $OKT8^+$ cells in asymptomatic thyroiditis. The correlation between the different T cells subsets and the values of FT4 I (when available) was investigated in the whole population of patients. While no correlation was found between $OKT3^+$ cells, $OKT4^+$ cells, $OKIa^+$ cells, $OKT4^+$ to $OKT8^+$ ratio and FT4 I values, a significant negative correlation was observed between $OKT8^+$ cells and FT4 I levels (Fig. 1). No correlation was found between $OKT8^+$ and $OKIa^+$ cells, or any kind of T cells subset and TGA titre.

Table 1. T lymphocyte subsets in patients with thyroid autoimmunity and in normal subjects, expressed in percentage of the controls (mean ± s.d.)

Condition	No. of subjects	Age mean (range)	Sex ratio (F:M)	OKT3$^+$	OKT4$^+$	OKT8$^+$	OKIa$^+$	OKT4$^+$:OKT8$^+$
Graves' disease (toxic)	6	52 (33–82)	5:1	80 ± 11¶	81 ± 11‡	63 ± 12¶†	168 ± 36¶†	1·33 ± 0·34
Graves' disease (euthyroid)	8	49 (16–70)	5:3	81 ± 20*	78 ± 32	88 ± 20	105 ± 59	0·96 ± 0·57
Myxoedema (hypothyroid)	9	56 (43–75)	8:1	103 ± 5§	87 ± 25	123 ± 36§	117 ± 58	0·80 ± 0·42§
Myxoedema (euthyroid)	12	67 (39–82)	12:0	88 ± 14*	94 ± 26	69 ± 26‡	105 ± 45	1·52 ± 0·65*
Asymptomatic thyroiditis (without medication)	12	43 (21–61)	11:1	93 ± 7*	95 ± 22	86 ± 22	131 ± 47	1·25 ± 0·74
Asymptomatic thyroiditis (on thyroxine)	8	44 (18–63)	8:0	95 ± 13	97 ± 12	84 ± 13*††	107 ± 48	1·17 ± 0·19
Hashimoto's goitre (without medication)	2	34 (29–38)	2:0	96 ± 1	87 ± 1	77 ± 36	103 ± 9	1·27 ± 0·42
Hashimoto's goitre (on thyroxine)	10	55 (41–66)	9:1	92 ± 10*	93 ± 28	99 ± 35‡‡	152 ± 61*	1·10 ± 0·64
Simple goitre with thyroiditis (without medication)	4	53 (32–68)	4:0	91 ± 10	96 ± 34	89 ± 32	119 ± 20	1·36 ± 0·90
Simple goitre with thyroiditis (on thyroxine)	4	58 (55–60)	3:1	91 ± 13	103 ± 30	74 ± 29	104 ± 34	1·64 ± 0·91
Normal control subjects	21	35 (21–50)	19:2	100 ± 12	100 ± 20	100 ± 26	100 ± 43	1·00 ± 0·58

* Compared to the control or † to the corresponding euthyroid group, $P < 0·05$.

‡ Compared to the control or § to the corresponding euthyroid group, $P < 0·01$.

¶ Compared to the control or to the corresponding euthyroid group, $P < 0·001$.

†† OKT8$^+$ cells in untreated and treated patients with asymptomatic thyroiditis: 85 ± 19*.

‡‡ Out of the 10 patients, two had OKT8$^+$ cells respectively of 160 & 158% for unknown reason (one had thalassemia minor) while the others had OKT8$^+$ cells lower than 100%.

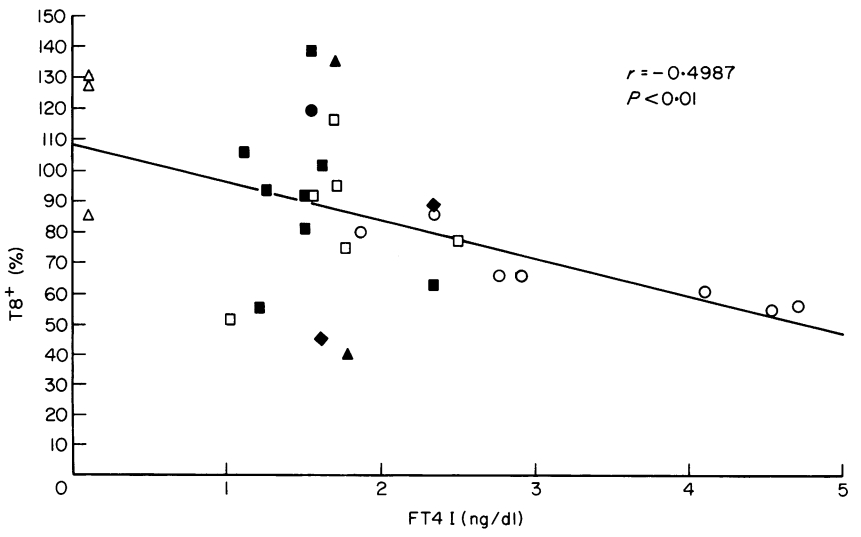

Fig. 1. Correlation between the percentage of circulating OKT8$^+$ cells and serum FT4I values in 29 patients with various autoimmune disorders. Graves' disease (toxic ○, euthyroid ●), myxoedema (hypothyroid △, euthyroid ▲), asymptomatic thyroiditis ■, Hashimoto's thyroiditis □, simple goitre with thyroiditis ♦.

DISCUSSION

In a recent study no significant difference was found in the proportion of OKT3$^+$, OKT4$^+$ and OKT8$^+$ cells between young and old women while only old men had modifications in these T cells subsets when compared with young men and with women (Mascart-Lemone *et al.*, 1982). Therefore, in the present study, the female to male ratio of 10:1 observed for all the patients was also obtained in the control subjects while no female subject older than 50 years was included in the control group.

Using functional assays, previous studies have shown a decrease in suppressor T lymphocyte activity in untreated Graves' disease (Aoki *et al.*, 1979; Okita *et al.*, 1981) and in Hashimoto's thyroiditis (Okita *et al.*, 1981). Other authors with similar techniques failed to find specific suppressor T cell abnormalities in Hashimoto's thyroiditis (Canonica *et al.*, 1981; Wall & Chartier, 1981; MacLean *et al.*, 1981) and in Graves' disease (Wall & Chartier, 1981; MacLean *et al.*, 1981). Recently, by enumeration of the T lymphocyte subsets using monoclonal antibodies of the OKT serie, a decrease in the percentage of peripheral T suppressor cells (OKT8$^+$) has been reported in Graves' disease (Thielemans *et al.*, 1981; Sridama *et al.*, 1982), in Hashimoto's goitre (Sridama *et al.*, 1982), in myxoedema and in asymptomatic thyroiditis irrespective of the clinical status of the patients (Thielemans *et al.*, 1981). Moreover, an activated state of circulating T lymphocytes and an increased number of helper T cells has been recently documented in Hashimoto's disease (Canonica *et al.*, 1982).

The results of our study are clearly confirming previously reported data showing abnormal distribution of circulating T cell subsets in patients presenting different types of thyroid autoimmune diseases. The most important finding involves a negative correlation between OKT8$^+$ cells and serum FT4 I showing that the serum T4 level can influence the percentage of circulating OKT8$^+$ cells, independently of the type of thyroiditis. This is in accordance with previous observations (Bonnyns *et al.*, 1978) showing that circulating lymphocytes from patients with Graves' disease had an increased *in vitro* responsiveness to mitogens directly related to serum T4 level. In view of the present study, the latter phenomenon can now be explained by a decreased

percentage of the OKT8$^+$ cells. Different mechanisms are probably involved in the regulation of the peripheral distribution of OKT8$^+$ cells. Indeed, in patients with myxoedema who had an increased percentage of OKT8$^+$ cells, the correction of the hypothyroidism by adequate thyroxine replacement doses induced a significant decrease in OKT8$^+$ cells in comparison with the percentage of suppressor cells in untreated myxoedema as well as in control subjects. This finding was confirmed by the significant increase in the OKT4$^+$ to OKT8$^+$ ratio in the group of patients with treated myxoedema. Thus in myxoedema, thyroxine treatment could facilitate peripheral redistribution of T cells and/or reveal a decreased production of these cells. In favour of the latter hypothesis, this trend to a decrease in the percentage of OKT8$^+$ cells was still observed in patients with Graves' disease after correction of the hyperthyroidism as well as in all the other groups of euthyroid thyroiditis patients.

The decrease of the total T lymphocytes (OKT3$^+$) in patients with Graves' disease and with myxoedema had to be interpretated as the results of the quantitative decline of the suppressor cells and perhaps of a relative increase in B cells since it is known that B cells bear Ia antigen.

The increased percentage of Ia$^+$ cells observed in patients with Graves' disease and with Hashimoto's goitre is in agreement with other reports (Canonica *et al.*, 1982; Jackson *et al.*, 1982). It may partly reflect the presence of activated T cells as Ia$^+$ T cells in normal subjects have been shown to increase after triggering of the immune system (Yu *et al.*, 1980). In Graves' disease the decreased proportion of OKT8$^+$ cells and the increased percentage of Ia$^+$ cells suggest an overstimulation of the lymphocytes and a possible peripheral trapping of OKT8$^+$ cells, a situation reversed under treatment. The peripheral trapping could also explain the decreased percentage of OKT4$^+$ cells in Graves' disease.

In conclusion, a common clear-cut profile of circulating T cell distribution is not emerging as a global characteristic feature for all our patients. This by itself documents the heterogeneity of factors that regulate circulating T cells in autoimmune thyroiditis. The exact significance of a decrease in the OKT8$^+$ cells remains under debate since OKT8$^+$ phenotype represents two distinct subsets of cells (suppressor and cytotoxic) and because the measurement of circulating lymphocytes cannot indicate the size of the lymphocyte pool. Therefore, any attempt to relate distribution of T cells to the immune status is premature. Moreover, even a decrease in the percentage of circulating suppressor cells cannot document a defect of specific suppression against any antigen.

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