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One-Year Prophylactic Treatment of Euthyroid Hashimoto's Thyroiditis Patients with Levothyroxine: Is There a Benefit?

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Studies in animal models of spontaneous Hashimoto's autoimmune thyroiditis (HT) show that prophylactic treatment with levothyroxine (LT_4) can reduce incidence and degree of lymphocytic infiltration in HT. The aim of the present study was to clarify whether there is a benefit of prophylactic treatment with LT_4 in patients with euthyroid HT with respect to the progression of the autoimmune process. Twenty-one patients with euthyroid HT were checked for thyroid function (thyrotropin [TSH], free triiodothyronine [FT_3], free thyroxine [FT_4]), thyroid volume, antibodies (thyroglobulin [Tg-Ab], thyroid peroxidase [TPO-Ab]), and lymphocyte subsets. Peripheral (PBL) and thyroid-derived lymphocytes (TL) were analyzed by triple color flow cytometry. One-half of the patients with euthyroid HT were treated with LT_4 for 1 year ($n = 10$). The other half ($n = 11$) were never treated with LT_4. TL were obtained by fine-needle aspiration biopsy (FNAB). Thirteen healthy subjects (C) without medical history of thyroid disease served as controls concerning PBL, and patients with nontoxic nodular goiter (NG; $n = 10$) served as controls concerning TL. Thyroid-derived T-helper cells were found more frequently in euthyroid patients with HT compared to patients with NG ($p < 0.01$). After 1 year of therapy with LT_4, TPO-Abs and B lymphocytes decreased significantly only in the treated group of euthyroid patients with HT ($p < 0.05$). In contrast, TPO-Abs levels did not change or even increased in untreated euthyroid patients with HT. Thyroid volume did not differ before and after therapy. Prophylactic treatment of euthyroid patients with HT reduced both serological and cellular markers of autoimmune thyroiditis. Therefore, prophylactic LT_4 treatment might be useful to stop the progression or even manifestation of the disease. However, the long-term clinical benefit of prophylactic LT_4 therapy in euthyroid patients with HT is yet to be established.

Introduction

SPONTANEOUS LYMPHOCYTIC THYROIDITIS has been described in several species of laboratory animals. Four of these models that spontaneously develop autoimmune thyroiditis gained certain importance in contributing to our understanding of organ-specific autoimmune disease in humans: the obese strain (OS) of chicken, the BioBreeding/Worcester (BB/Wor) rats, the spontaneous thyroiditis in cats, and the nonobese diabetic (NOD) mouse (1). In OS chicken, levothyroxine (LT_4) at thyrotropin (TSH) suppressive doses could induce a slight amelioration of thyroiditis. The combination of TSH-suppressive LT_4 therapy with severe iodine deficiency was able to almost completely inhibit the autoimmune process in this species. The effect of long-term TSH-suppressive therapy in BB/Wor-rats developing spontaneous lymphocytic thyroiditis significantly decreases the incidence of autoimmune thyroiditis, and in addition, decreases the serum levels of thyroglobulin autoantibodies. In genetically predisposed cats, early treatment with LT_4 was followed by a significant reduction in the incidence of the disease as assessed by typical features of thyroiditis (intensity of lymphocytic infiltration, HLA expression of thyroid cells of cat thyroid, serological markers). The dose of LT_4 did not appear to be excessive either in BB/Wor-rats or in cats.

Our present study highlights data that evaluate the effect of thyroid hormones (LT_4) in thyroid autoimmune disease both, spontaneous animal thyroiditis and human autoimmune disease.

Hashimoto's thyroiditis (HT), the most common form of autoimmune thyroid disease (AITD), is characterized by lymphocytic infiltration of the thyroid gland, gradual destruction of the organ, and production of thyroid-specific autoantibodies (antithyroid peroxidase [TPO-Ab] and antithyroglobulin [Tg-Ab] antibodies). Due to the intrathyroidal important role of T-lymphocytes' in the pathogenesis of AITD (2), subsets of thyroid-derived lymphocytes are useful to determine the activity of the autoimmune process.

Cellular destruction and ultimately hypothyroidism are caused by various humoral and cellular mechanisms of au-

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toimmunity. Recently it has been discussed that the Fas-mediated apoptosis of thyrocytes after interleukin-β (IL-β) exposure (3,4) could play a major pathogenic role.

The decrease of serum thyroid autoantibodies (TPO-Ab and Tg-Ab) during LT_4 therapy is still controversial. Previous studies in hypothyroid patients with HT before and after LT_4 therapy showed a decrease of Tg-Ab and TPO-Ab levels (5–7), whereas this change was not observed by other authors (8,9).

In models of spontaneous thyroiditis, early prophylactic LT_4 treatment with subsequent low thyrotropin (TSH) levels reduced severity and changed the time course in genetically predisposed animals such as cats, BB/Wor-rats, and OS-chicken (10–12).

The goal of the present clinical study was to investigate whether the experimental data in animal models of spontaneous HT are matched by similar findings in patients with HT. We evaluated the influence of prophylactic LT_4 therapy in euthyroid patients with HT, and studied the influence of LT_4 treatment on serum Tg-Ab and TPO-Ab in these patients. Furthermore, thyroid-derived lymphocytic subsets in euthyroid patients with HT were characterized.

Materials and Methods

Patients

Blood samples were obtained after written informed consent from 21 patients with HT (20 women and 1 man; age range, 37–51 years, median age 41 years) and 13 healthy subjects (10 women, 3 men; age range, 24–58 years, median age, 34 years) who had no family history of AITD who served as controls. The diagnoses of HT (euthyroid) was based on ultrasonography, laboratory criteria (thyrotropin [TSH], free triiodothyronine, free thyroxine [FT_4], Tg-Ab, TPO-Ab), and cytology. Patients and control subjects were examined with respect to thyroid function (TSH, FT_3, FT_4 [Automatic Chemiluminescence System, ACS 180, Chiron, Fernwald, Germany]), thyroid volume, echo pattern, and antibodies (Tg-Ab, TPO-Ab [Elias, Freiburg, Germany]). Thyroid size was determined by ultrasound, before and at the end of the observation period while receiving LT_4 using a Toshiba SSA-340A apparatus with a transductor of 7.5 MHz. The total thyroid volume was calculated from the sum of the partial volumes (right lobe plus left lobe). The volume of every part was calculated by means of the formula V = length $\times$ width $\times$ thickness $\times$ 0.5. Normal values for one part were 9 to 12.5 mL.

The 21 euthyroid patients with HT were divided into two groups. None of the patients had any symptoms of hypothyroidism nor did they have abnormal thyroid volume. The patients were all euthyroid and the diagnosis was the result of routine examination. The patients were divided into two groups alternately immediately after diagnosis. The diagnosis of euthyroid HT was made in all 21 patients just before they entered the study. No data exist of prior examinations.

One group (group 1, treated HT) included 10 patients who underwent fine-needle aspiration biopsy (FNAB) before and after treatment (1 year) with LT_4 and 11 patients (group 2, untreated HT) who were not treated and did not have a second FNAB. Group 1 was treated for a median period of 12 months with LT_4 with a daily dose ranging from 1.0 to 2.0 μg/kg of LT_4 per day. The aim of LT_4 therapy was to decrease TSH to the lower normal range (0.3–1.0 μU/mL). The therapy was initiated in all patients with 50 μg of LT_4 per day. After 4 weeks the level of serum TSH was checked and the dose of LT_4 was adjusted to reach the range of a TSH level of 0.3–1.0 μU/mL. None of the patients received a TSH suppressive dose of LT_4. A FNAB was taken before and after 1 year of prophylactic treatment with LT_4 from 10 patients with euthyroid HT. After every FNAB all cells were counted and checked for viability. The viability was always above 98%. The amount of cells we counted differed from 500.000 to 3,000,000 cells per aspiration. For the staining of cells we took samples of 50,000 cells in 100 μL for thyroid derived cells and 1×10^6 cells for peripheral lymphocytes. Using the FACS-scan method the amount of the subpopulation (here, B cells) are expressed as percentages of the total amount of lymphocytes.

None of the patients with HT ever received replacement therapy with synthetic LT_4 before the time of sampling. All 21 patients with HT (100%) were positive for TPO-Ab (TPO-Ab $>$ 100 U/mL), whereas only 16 of 21 HT patients (76%) had elevated Tg-Ab levels (Tg-Ab $>$ 100 U/mL) (Table 1). In

Table 1. Survey of Patients (n = 31) and Controls (n = 13): Median Age, Range of Age, and Autoantibodies (TPO-Ab, Tg-Ab)

Patients and controls	*n*	*Age*	*Elevated TPO-Ab*	*Elevated Tg-Ab*
Euthyroid patients with HT (EU)	21	41 (37–51)	21/21 (100%)	16/21 (76%)
will be treated with LT_4, for 1 year (group 1, data from beginning of the study before treatment)	*10*	*40 (36–54)*	*10/10 (100%)*	*8/10 (80%)*
will not be treated with LT_4 (group 2, data from beginning of the study)	*11*	*37 (34–47)*	*11/11 (100%)*	*8/11 (73%)*
Patients with nontoxic nodular goiter (NG)	10	49 (29–77)	0/10 (0%)	0/10 (0%)
Healthy controls	13	34 (24–58)	0/13 (0%)	0/13 (0%)

Cut off level for TPO-Ab and Tg-Ab $<$ 100 U/mL.
TPO-Ab, thyroid peroxidase antibodies; Tg-Ab, thyroglobulin antibodies; HT, Hashimoto's thyroiditis.

order to compare the thyroid-derived lymphocyte subsets from patients with autoimmune HT with those of a nonautoimmune thyroid disease, thyroid tissue from 10 patients with nontoxic nodular goiter (NG) (5 women and 5 men; age range, 29–77 years, median 49 years) who underwent surgery for a large goiter was obtained at surgery (sample size of 5 g) after giving informed consent (Table 1). Patients with NG and controls were euthyroid and had no antithyroid autoantibodies.

Prophylactic LT_4 therapy

All 10 patients with euthyroid HT who had never been treated with thyroid hormones received LT_4 immediately after FNAB. The aim of LT_4 therapy was to reduce TSH to the lower normal range (0.3–1.0 μU/mL). The patients were treated for a median period of 1 year with LT_4 with daily doses of 1.0 to 2.0 μg/kg of LT_4 per day. The protocol of this study was approved by the ethics committee of the hospital.

Cell separation

Blood samples were collected at 8:00 AM from both patients and healthy subjects in order to minimize circadian variations of lymphocyte subsets. peripheral blood mononuclear cells (PBMC) obtained from heparinized venous blood were isolated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation. Cell viability of more than 98% was achieved routinely as assessed by trypan blue (Gibco, Karlsruhe, Germany) Cells were washed three times with phosphate-buffered saline (PBS, Gibco). FNAB was carried out in patients with euthyroid HT at the same time of day; thyroid tissue was obtained during surgery from patients with NG at variable times of the day. The thyroid tissue was cut into small pieces using a tissue chopper (McIlwain Tissue Chopper, Mickle Laboratory Engineering Co. LTD., England), and rinsed in PBS. The suspension, including intrathyroidal lymphocytes, was filtered through different sized nylon meshes (100 μm, 70 μm, 40 μm, and 35 μm; Becton Dickinson, Heidelberg, Germany). Finally, the thyroid-derived lymphocytes were isolated by density gradient centrifugation.

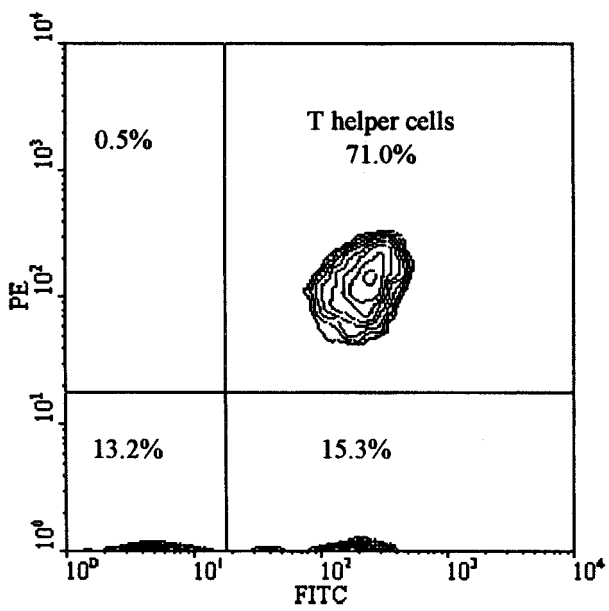

FIG. 1. Thyroid-derived T-helper cells (CD45/CD3/CD4) from a patient with Hashimoto's thyroiditis. T-helper cells: 71.0%, $p < 0.05$ compared to T-helper cells in nontoxic nodular goiter.

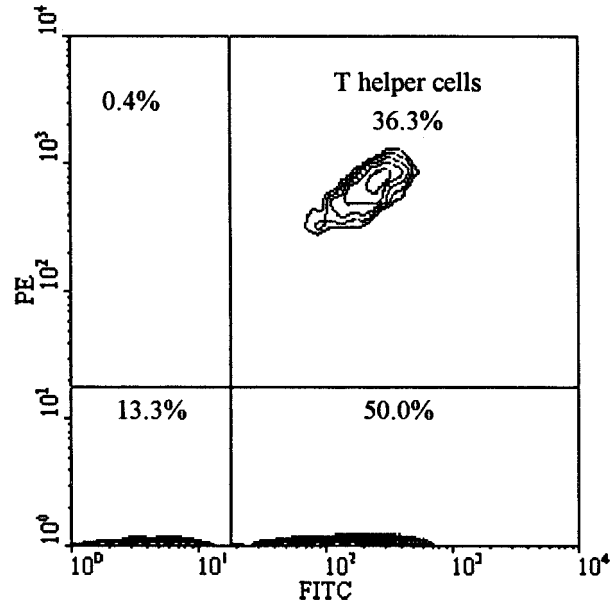

FIG. 2. Thyroid-derived T-helper cells (CD45/CD3/CD4) from a patient with nontoxic nodular goiter. T-helper cells: 36.3%, $p < 0.05$ compared to T-helper cells in Hashimoto's thyroiditis.

Cell staining and flow cytometry

Three-color flow cytometry (fluorescein isocyanate conjugated [FITC]-, PE-, and PerCP-labeled mononuclear antibodies; Becton Dickinson Immunocytometry Systems, Heidelberg, Germany) was performed to determine the following lymphocyte subsets: $CD3^+/CD4^+$ (T-helper cells), $CD3^+/CD8^+$ (cytotoxic T cells), $CD3^-/CD19^+$ (B lymphocytes), $CD3^-/CD56^+$ (natural killer cells), $CD4^+/HLA\text{-}DR^+$ (activated T-helper cells), $CD8^+/HLA\text{-}DR^+$ (activated cytotoxic T cells). For this purpose, 100-μL samples of mononuclear cells (1×10^6 cells for peripheral lymphocytes, 50,000 cells for thyroid-derived lymphocytes) were incubated for 15 minutes at room temperature in the dark with a combination of 20 μL of monoclonal antibodies conjugated with FITC, PE, or PerCP. All analyses were performed with a FACScan flow cytometer (Becton Dickinson) that had been calibrated with CaliBRITE beads and AutoCOMP software (Becton Dickinson). The relative size of each lymphocyte subset was expressed as the percentage of the total lymphocyte population.

Statistics

Statistical analyses were performed by Wilcoxin test, Mann-Whitney test, Spearman rank correlation coefficient, and analysis of variance. The Mann-Whitney test was used to analyze PBL from controls and from patients with HT. The correlation between the number of thyroid-derived cells,

Table 2. Thyroid Hormones and Antithyroid Antibodies in Patients with Hashimoto's Thyroiditis Before and After One Year of Prophylactic Therapy with LT_4[a]

	Euthyroid patients with HT with LT_4 treatment		*Euthyroid patients with HT without LT_4 treatment*	
	Before therapy	*After therapy*	*At time of diagnosis*	*After one year of diagnosis*
TPO-Ab	759	520*	1328	1680
(<100 U/mL)	(624.3; 1349)	(325.8; 686)	(530.5; 3000)	(586.5; 3000)
Tg-Ab	147	86	262	315
(<100 U/mL)	(77; 1016.5)	(43; 620.5)	(81; 462)	(169.5; 808)
TSH	2.9	0.7*	2.0	2.4
(0.3–4.5 μU/mL)	(2.35; 3.3)	(0.275; 1.3)	(1.85; 2.25)	(1.85; 2.9)
FT4	1	1.4*	1.2	1.2
(0.8–1.8 ng/dL)	(0.85; 1.18)	(1.3; 1.6)	(1.1; 1.35)	(1.1 1.35)

[a]The values are expressed as median (1./3. quartiles).
*$p < 0.05$.
TPO-Ab, thyroid peroxidase antibodies; Tg-Ab, thyroglobulin antibodies; TSH, thyrotropin; FT_4, free thyroxine; LT_4, levothyroxine.

antibody levels, hormone levels, and thyroid size was tested according to the method of Spearman. Data are expressed as medians and 1./3. quartiles. A p value < 0.05 was considered statistically significant.

Results

Peripheral lymphocyte subsets in patients with HT

Six lymphocyte subsets (T-helper cells, cytotoxic T cells, B lymphocytes, natural killer cells, activated T-helper cells, activated cytotoxic T cells) from peripheral blood of patients with HT were compared with subsets from control subjects. No differences were observed. Also no differences were seen comparing group 1 and group 2 of patients with euthyroid HT.

Thyroid-derived lymphocyte subsets in patients with HT

Thyroid-derived T-helper cells were found more frequently in euthyroid patients with HT ($p < 0.01$) compared to patients with NG (Figs. 1 and 2). All other lymphocyte subsets did not differ between patients with HT and NG.

Prevalence and level of TPO-Ab and Tg-Ab in HT

All 21 patients with HT were positive for TPO-Ab before treatment. In contrast, Tg-Ab were detected in 16 of 21 (76%) euthyroid patients (Table 1).

Levels of TPO-Ab titers were higher (median TPO-Ab, group 1: 759 U/mL; group 2: 1328 U/mL) compared to Tg-Ab (median Tg-Ab, group 1: 147 U/mL; group 2: 262 U/mL) (Table 2).

Comparison of TPO-Ab, Tg-Ab, thyroid volume, and TSH

Thyroid antibody titers (TPO-Ab, Tg-Ab) did not correlate with thyroid volume or with TSH in all 21 patients with HT (before and after 1 year with or without LT_4 therapy).

Effect of LT_4 treatment

After 1 year of LT_4 therapy (group 1) the serum FT_4 levels increased significantly (median FT_4, from 1.0 to 1.4 ng/dL) whereas the serum TSH levels decreased significantly in this group (median TSH, from 2.9 to 0.7 μU/mL) (Table 2, Fig. 3).

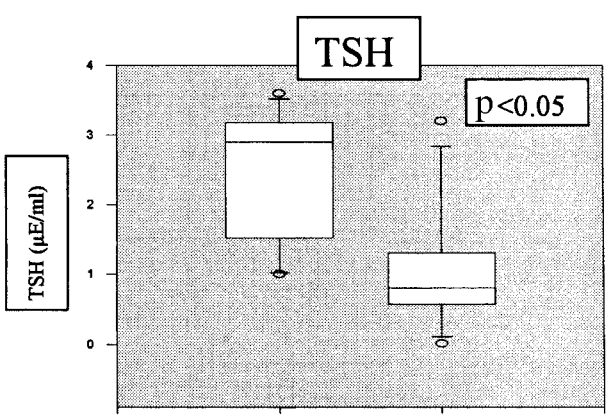

FIG. 3. Thyrotropin (TSH) before and after therapy with levothyroxine ($p < 0.05$).

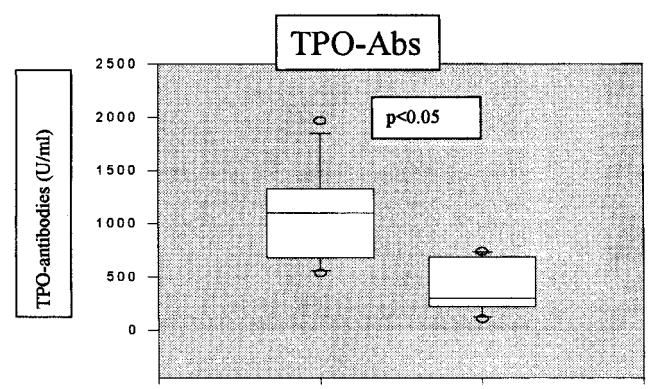

FIG. 4. Thyroid peroxide antibodies (TPO-Abs) before and after 1 year of therapy with levothyroxine ($p < 0.05$).

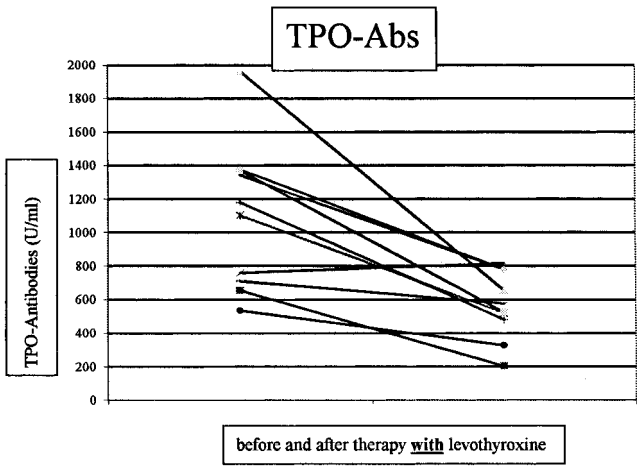

FIG. 5. Thyroid peroxidase antibodies (TPO-Abs) in euthyroid patients with euthyroid Hashimoto's thyroiditis before and after 1 year of prophylactic treatment with levothyroxine ($p < 0.05$).

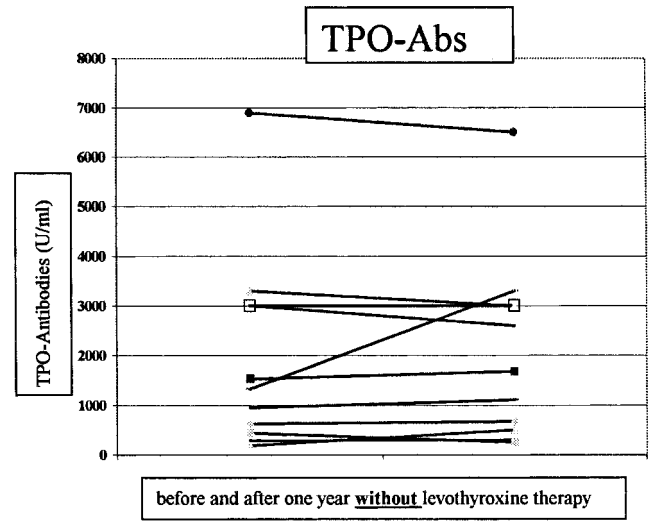

FIG. 7. Thyroid peroxidase antibodies (TPO-Abs) in euthyroid patients with euthyroid Hashimoto's thyroiditis before and after 1 year without levothyroxine therapy (p = NS).

In addition the TPO-Ab titers decreased significantly in patients with euthyroid HT (P = 0.01; median TPO-Ab, from 759 to 520 U/mL) (Table 2, Fig. 4 and 5). Tg-Ab also decreased during LT_4 therapy, however, without approaching the level of statistical significance. (p = NS; median Tg-Ab: 147 to 86 U/mL) (Table 2, Fig. 6).

In contrast, TPO-Ab and Tg-Ab did not decrease significantly in untreated patients with euthyroid HT (group 2) (Fig. 7).

Thyroid-derived B lymphocytes also decreased significantly in euthyroid patients with HT after 1 year of LT_4 treatment ($p < 0.05$, Fig. 8). No other lymphocyte subset other than B lymphocytes changed after 1 year of LT_4 treatment.

Discussion

Autoimmune diseases are characterized by an organ-specific and damaging response of the immune system directed against autoantigens (13). In chronic autoimmune thyroiditis the thyroid gland is infiltrated by B and T lymphocytes that are responsible for cellular destruction and the formation of thyroid-specific antibodies (14,15). A major site of antibody production are the lymphocytes in the thyroid gland itself (16).

Peripheral and thyroid-derived lymphocytes

Activation of thyroid antigen-specific T-helper lymphocytes is considered to be one of the earliest steps in the autoimmune process of HT (17). The mechanism of their activation is still unclear. The activated, self-reactive T-helper cells can induce a B-cell reaction, resulting in migration of these cells into the thyroid gland and secretion of antibodies against thyroid-specific antigens (18). The results of our study demonstrate an increased percentage of thyroid-derived T-helper cells in euthyroid patients with HT compared to thyroid-derived lymphocytes of patients with NG. This immune phenomenon could reflect the active inflammatory process of HT in the thyroid gland.

In contrast to previous reports (19), in our study no difference between PBL subsets derived from patients with HT

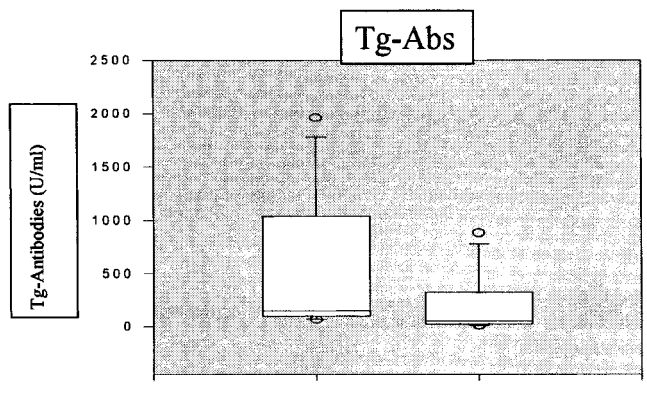

FIG. 6. Thyroglobulin antibodies (Tg-Abs) before and after 1 year of therapy with levothyroxine (p = NS).

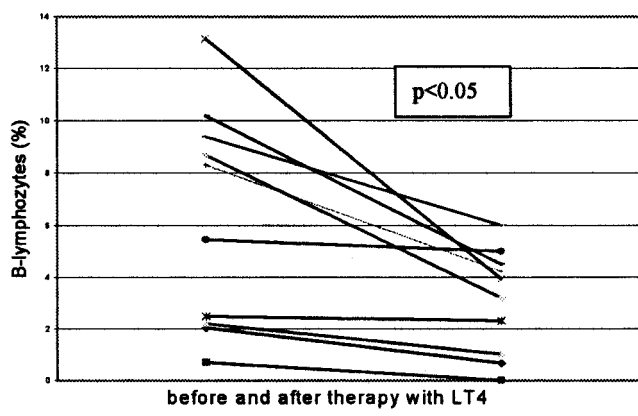

FIG. 8. Thyroid-derived B lymphocytes in euthyroid patients with Hashimoto's thyroiditis before and after 1 year of prophylactic treatment with levothyroxine ($p < 0.05$).

and healthy control subjects was observed. Similar to our findings no differences in PBL subsets were observed in untreated patients with GD, HT, and malignant lymphoma (20). This is in keeping with the fact that HT is an organ-specific autoimmune disease (2), and that our data do not show characteristics of a systemic disorder with a generalized clinical manifestation.

Prophylactic LT_4 therapy in still euthyroid patients

As previously shown in animal models of spontaneous lymphocytic thyroiditis (OS-chicken, BB-rats, cats), prophylactic thyroxine therapy influences incidence, time of disease manifestation, and lymphocytic infiltration into the thyroid (12,21).

In addition, the uniform pattern of infiltrating lymphocyte subsets derived from thyroid glands of euthyroid patients provide strong evidence that a prophylactic LT_4 therapy in euthyroid patients could be effective and reduce the autoimmune activity. The mechanism for this reduction could be the reduced antigen availability of the immune system that is probably due to a reduced thyroid stimulation after decreasing TSH levels (6). We could show that in patients with euthyroid HT who did not undergo prophylactic treatment with LT_4, TSH and antibody titers remain the same whereas in patients who received prophylactic treatment, TSH and antibody titers decrease significantly.

The influence of LT_4 treatment and consecutive low TSH serum levels on thyroid autoimmune activity in patients with HT and Graves' disease has been studied by several authors. Most of the studies particularly investigated the relationship of thyroid function, autoantibody levels, and thyroid volume. Contradictory data were reported: although some clinical studies found a decrease of TPO-Ab and/or Tg-Ab levels in adult patients with HT during LT_4 therapy (6,7,22,23), others could not confirm these data (5,8,9).

Our study has evaluated the influence of prophylactic LT_4 treatment on autoantibody levels in euthyroid patients with HT. In support of a potentially beneficial effect of prophylactic LT_4 treatment is our finding that TPO-Ab are significantly reduced after 12 months of treatment whereas no change in antibody titers were observed in untreated patients with euthyroid HT.

As a characteristic serological autoimmune phenomenon high titers of thyroid specific antibodies were found ($>$ 100% TPO-Ab, 76% Tg-Ab) in the sera of patients with HT. In other studies elevated Tg- and TPO-Ab were observed in 74% and 96% respectively in patients with histological assured thyroiditis (18,24–27).

Of interest in this context is the study of Chiovato et al. (28) who describe—similar to our study—a significant decrease of thyroid antibodies in patients with hypothyroid autoimmune thyroiditis treated with LT_4. In contrast to our findings (which demonstrated a significant decrease in TPO-Ab in euthyroid and hypothyroid patients with HT) Chiovato et al. (28) observed a decrease only in hypothyroid patients. The difference between the study by Chiovato et al. (28) and ours might result because of different methods used to measure the antibodies. Chiovato et al. (28) measured thyroid microsomal and thyroglobulin antibodies by sensitive and quantitative radioassays in goitrous patients with HT whereas antibodies in our study were measured by enzyme-linked immunosorbent assay (ELISA).

It is still unknown if the disease-specific antibodies (TPO-Ab and Tg-Ab) are directly involved in the pathogenesis of the disease or if the antibodies are just secondary to tissue destruction by thyroid infiltrating T-lymphocytes (29,30). The influence of different antibody populations on thyroid function in patients with HT is controversial (31–34). It is not yet clear whether TPO-Abs are a serological feature of the intrathyroidal autoimmune process or if they are able to induce functional changes, e.g., hypothyroidism by blocking the enzyme TPO (18,24). A major group of authors postulate a correlation of lymphocytic infiltration and the activity of the disease process with TPO-Ab-titers (32,35,36). In contrast, Kasagi and coworkers (27) suggest Tg-Ab as the crucial pathogenetic event. Methodological differences in the determination of serum Tg-Ab and TPO-Ab levels may contribute to these controversial discussion. In our study TPO-Abs were a better predictor for thyroid autoimmune disease compared to Tg-Ab. These antibodies were not only found more frequently than Tg-Ab (100% vs. 62%) but also TPO-Ab titers were higher in euthyroid patients with HT compared to Tg-Ab. Other authors could also show that in postpartum thyroid dysfunction (PPTD), high TPO-Ab levels and a hypoechogenic ultrasound pattern lead to a high risk of long-term thyroid dysfunction (37).

In summary, an ongoing autoimmune process in the early stages of euthyroid patients with HT was demonstrated in our study. The prophylactic treatment of euthyroid patients with HT for 1 year reduced TPO-Ab titers significantly. Further studies should provide insight concerning the underlying mechanisms and establish the clinical benefit of prophylactic LT_4 treatment.

References

1. Schumm-Draeger P-M, Wenzel BE 1996 In vivo models in thyroid research. Exp Clin Endocrin Diabetes **104**(Suppl 3):1–63.
2. Weetman AP 1996 Chronic autoimmune thyroiditis. In: Braverman LE, Utinger RD, eds. Werner and Ingbar's The Thyroid, 7th Edition. Lippincott-Raven Publishers, Philadelphia, pp. 738–748.
3. Giordano C, Stassi G, De Maria R, Todaro M, Richiusa P, Papoff G, Ruberti G, Bagnasco M, Testi R, Galuzzo A 1997 Potential involvement of Fas and ist ligand in the pathogenesis of Hashomoto's thyroiditis. Science **257**:960–963.
4. Williams N 1997 Thyroid disease: A case of cell suicide? Science **275**:926.
5. Hegedus L, Hansen JM, Feldt-Rasmussen U, Hansen BM, Hoier-Madsen M 1991 Influence of thyroxine treatment on thyroid size and anti-thyroid peroxidase antibodies in Hashimoto's thyroiditis. Clin Endocrinol (Oxf) **35**:235–238.
6. Rieu M, Richard A, Rosilio M, Laplanche S, Ropion V, Fombeur JP, Berrod JL 1994 Effects of thyroid status on thyroid autoimmunity expression in euthyroid and hypothyroid patients with Hashimoto's thyroiditis. Clin Endocrinol (Oxf) **40**:529–535.
7. Romaldini JH, Biancalana MM, Figueiredo DI, Farrah CS, Mathias PC 1996 Effect of L-thyroxine administration on antithyroid antibody levels, lipid profile, and thyroid volume in patients with Hashimoto's thyroiditis. Thyroid **6**:183–188.
8. Hayashi Y, Tamai H, Fukata S, Hirota Y, Katayama S, Kuma K, Kumagai LF, Nagataki S 1985 A long term clinical, immunological, and histological follow-up study of patients with goitrous chronic lymphocytic thyroiditis. J Clin Endocrinol Metab **61**:1172–1178.

9. Papapetrou PD, MacSween RN, Lazarus JH, Harden RM 1972 Long-term treatment of Hashimoto's thyroiditis with thyroxine. Lancet **2**:1045–1048.
10. Reinhardt W, Paul TL, Allen EM, Alex S, Yang YN, Appel M, Braverman LE 1988 Effect of L-Thyroxine administration on the incidence of iodine induced and spontaneous lymphocytic thyroiditis in the BB/Wor rat. Endocrinology **122**:1179–1181.
11. Allen EM and Braverman LE 1996 The biobreeding worcester rat-a model of organ-specific autoimmunity. Exp Clin Endocrinol Diabetes **104**(Suppl 3):7–10.
12. Schumm-Draeger P-M, Fortmeyer HP 1996 Autoimmune thyroiditis—Spontaneous disease models—cat. Exp Clin Endocrinol Diabetes **104**(Suppl 3):12–13.
13. Jones DE, Diamond AG 1995 The basis of autoimmunity: An overview. Baillieres Clin Endocrinol Metab **9**:1–24.
14. Eguchi K, Matsuoka N, Nagataki S 1995 Cellular immunity in autoimmune thyroid disease. Baillieres Clin Endocrinol Metab **9**:71–94.
15. Cihak J, Hoffmann-Fezer G, Wasl M, Merkle H, Kaspers B, Vainio O, Plachy J, Hala K, Wick G, Stangassinger M, Losch U 1998 Inhibition of the development of spontaneous autoimmune thyroiditis in the obese strain (OS) chickens by in vivo treatment with anti-CD4 or anti-CD8 antibodies. J Autoimmun **11**:119–126.
16. Iwatani Y, Hidaka Y, Matsuzuka F, Kuma K, Amino N 1993 Intrathyroidal lymphocyte subsets, including unusual CD4+ CD8+ cells and CD3loTCR alpha beta lo/-CD4-CD8- cells, in autoimmune thyroid disease. Clin Exp Immunol **93**:430–436.
17. Weetman AP, McGregor AM 1994 Autoimmune thyroid disease: Further developments in our understanding. Endocr Rev **15**:788–830.
18. Dayan CM, Daniels GH 1996 Chronic autoimmune thyroiditis. N Engl J Med **335**:99–107.
19. Gessl A, Wilfing A, Agis H, Steiner G, Czernin S, Boltz-Nitulescu G, Vierhapper H, Waldhausl W 1995 Activated naive CD4+ peripheral blood T cells in autoimmune thyroid disease. Thyroid **5**:117–125.
20. Fujikawa M, Okamura K, Sato K, Mizokami T, Tanabe S, Ikenoue H, Okamura S, Ohta M, Fujishima M 1998 Usefulness of surface phenotype study of intrathyroidal lymphocytes obtained by fine needle aspiration cytology in autoimmune thyroid disease and malignant lymphoma of the thyroid. Clin Endocrinol **49**:191–196.
21. Wick G 1996 The role of the target organ in the development of autoimmune diseases exemplified in the obese strain (OS) chicken model for human Hashimoto disease. Exp Clin Endocrinol Diabetes **104**(Suppl 3):1–4.
22. Jansson R, Karlsson A, Dahlberg PA 1985 Thyroxine, methimazole, and thyroid microsomal autoantibody titres in hypothyroid Hashimoto's thyroiditis. Br Med J **290**:11–12.
23. Mariotti S, Loviselli A, Cambosu A, Velluzi F, Atzeni F, Martino E, Bottazzo GF 1996 The role of iodine in autoimmune thyroid disease in humans. In: The Thyroid and Iodine Merck, European Thyroid Symposium, Schattauer & Stuttgart, eds. New York: Warsaw 1996, May 16–18, pp. 155–168.
24. DeGroot LJ, Quintans J 1990 The causes of autoimmune thyroid disease. Endocr Rev **10**:537–562.
25. Rapoport B 1991 Pathophysiology of Hashimoto's thyroiditis and hypothyroidism. Ann Rev Med 42:91–96.
26. Singer PA 1991 Thyroiditis: Acute, subacute, and chronic. Med Clin North Am **75**:61–77.
27. Kasagi K, Kousaka T, Higuchi K, Iida Y, Misaki T, Alam MS. Miyamoto S, Yamabe H, Konishi J 1996 Clinical significance of measurements of antithyroid antibodies in the diagnosis of Hashimoto's thyroiditis: comparison with histological findings. Thyroid **6**:445–450.
28. Chiovato L, Marcocci C, Mariotti S, Mori A, Pinchera A 1986 L-thyroxine therapy induces a fall of thyroid microsomal and thyroglobulin antibodies in idiopathic myxedema and in hypothyroid, but not in euthyroid Hashimoto's thyroiditis. J Endocrinol Invest **9**:299–305.
29. Tomer Y 1997 Anti-thyroglobulin autoantibodies in autoimmune thyroid diseases: Cross-reactive or pathogenic? Clin Immunol Immunopathol **82**:3–11.
30. McIntosh RS, Asghar MS, Weetman AP 1997 The antibody response in human autoimmune thyroid disease. Clin Sci (Colch) **92**:529–541.
31. Bryant WP, Bergert ER, Mukuta T 1995 Identification of thyroid blocking antibodies and receptor epitopes in autoimmune hypothyroidism by affinity purification using TSH receptor peptides. Autoimmunity **22**:69–79.
32. Scherbaum WA, Paschke R 1995 Significance of thyroid antibodies for diagnosis and follow-up of thyroid diseases. Internist (Berl) **36**:303–309.
33. Heufelder AE, Hofbauer LC 1998 Die Thyreoiditiden 1998: Aktueller Stand der Pathogenese, Diagnostik und Therapie. Dt Ärzteblatt **95**:466–476.
34. Schumm-Draeger P-M 1998 Thyreoditis. Formen, Diagnostik, Therapie. Internist (Berl) **39**:594–598.
35. Engler H, Riesen WF, Keller B 1992 Diagnostic value of autoantibodies against microsomal thyroid peroxidase (anti-TPO). Schweiz Med Wochenschr **122**:1976–1980.
36. Nordyke RA, Gilbert FIJ, Miyamoto LA, Fleury KA 1993 The superiority of antimicrosomal over antithyroglobulin antibodies for detecting Hashimoto's thyroiditis. Arch Intern Med **153**:862–865.
37. Premawardhana LDKE, Parkes AB, Ammari F, John R, Darke C, Adams H, Lazarus JH 2000 Postpartum thyroiditis and long-term thyroid status: Prognostic Influence of thyroid peroxidase antibodies and ultrasound echogenicity J Clin Endocrinol Metab **85**:71–75.

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