Thyroid hormone autoantibodies in primary Sjögren syndrome and rheumatoid arthritis are more prevalent than in autoimmune thyroid disease, becoming progressively more frequent in these diseases

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ABSTRACT. To verify the greater prevalence of circulating thyroid hormone autoantibodies (THAb) in primary Sjögren syndrome (SS) *vs* Hashimoto's thyroiditis (HT) and Graves' disease (GD), we measured THAb in the serum of patients with these 3 diseases who were sampled from 1998-1999 (no.=20, 88, 25) and 1990-1992 (no.=13, 75, 31). Patients with rheumatoid arthritis (RA) (no.=23 and 16) and other collagenoses (no.=20 and 16) were also studied. A third series of patients with these 5 diseases was studied from 1975-1982, and data have been taken into account. THAb were detected using a specific radioimmunoprecipitation method, and their presence was correlated with the presence of TG antibodies (TGAb). We found that IgG antibodies against T_3, T_4 or both were present with these prevalences in the 1975-1982, 1990-1992 and 1998-1999 series: HT=1, 4, 20%; GD=2, 6, 32%; SS=20, 31, 50%; RA=0, 12, 26%; other collagenoses=0, 0, 0%. The majority of the Sjögren or arthritis cases positive for THAb were negative for TGAb, while the opposite was true for the 2 autoimmune thyroid diseases. We conclude that prevalence of THAb in the 2 non-thyroid autoimmune diseases is greater than in the 2 thyroid autoimmune diseases. In addition, prevalence of THAb is increasing over time regardless of disease. Molecular similarity between extra-thyroid connective proteins (specifically associated to primary SS and RA) and iodinated regions of TG, and an increased preponderance of environmental factors as triggers of autoimmune diseases might account for our findings. (J. Endocrinol. Invest. *25:* 447-454, 2002)

INTRODUCTION

Thyroid hormone autoantibodies (THAb) are considered the rarest circulating thyroid Ab (1). According to a European survey (2), the overall prevalence of THAb among consecutive patients attending thyroid clinics is 149/369,000 or 0.04%. Not surprisingly, THAb are relatively more frequent (<10%, and as low as 1%) in autoimmune thyroid diseases [Hashimoto's thyroiditis (HT) and Graves' disease (GD)] (1). THAb are virtually always of the immunoglobulin G (IgG) class, because this is the class typically sought (1). As to the hormone specificity, THAb against T_3 (T_3Ab), against T_4 (T_4Ab) and against both (T_3 T_4Ab) account for 51%, 28% and 21% of the cases, respectively (2), which is in agreement with the literature (1). Only approximately 50% of the THAb positive ($THAb^+$) cases are TG Ab positive ($TGAb^+$), presumably because most of the authors used the passive agglutination technique (1), not a very sensitive method for measuring TGAb (3).

On sera from patients with GD or HT collected over 1975-1982, prevalence of IgG-THAb was 1.8% or 0.8%, respectively (4). On sera from patients with autoimmune diseases of the connective tissue (collagenoses) collected over the same period of time, prevalences of IgG-THAb were: 20% [primary Sjögren syndrome (SS)], 0% [rheumatoid arthritis (RA)] and 0% [systemic lupus erythematous (SLE)] (4). In a subsequent and unpublished study (but data will be presented here), we measured IgG-THAb on sera collected in

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1990-1992 from patients with GD, HT, SS, RA and other collagenoses. Except for this last condition, in which THAb were absent, the frequency of THAb was greater than found in our previous series (4), with the following order: SS >RA >GD >HT. Shortly afterwards, a Mexican study published in 1995 (5) on 33 patients with primary SS reported an unsuspectedly high prevalence of THAb (16/33 or 48%), much greater than prevalence of TGAb (6/33 or 18%), and with the following hierarchy in thyroid hormone specificity: T_3 T_4Ab (62.5%) >T_4Ab (25%) >T_3 Ab (12.5%). However, they used a non-specific method of immunoprecipitation, in that polyethylene glycol precipitates several proteins and cannot distinguish Ig classes (1). In addition, they also used 8-anilino-naphtalene sulfonic acid, which is an inhibitor of thyroid hormone binding to the plasma proteins, and therefore its use may overestimate THAb (1).

To verify that THAb are unexpectedly more frequent in SS and RA *vs* GD and HT, we collected a third series of patients during 1998-1999 and measured THAb of both the IgG and the IgM class. The data presented are not only confirmatory but show that over the years THAb are becoming more frequent, regardless of disease.

PATIENTS AND METHODS

Patients

To ease comparison, the major characteristics of the 3 chronological series of patients are summarized in Table 1. Patients were always consecutive subjects admitted to the divisions of Endocrinology or to the 2 Institutes of Internal Medicine or to the Institute of Ophthalmology of our University Hospital, except approximately 15% of the GD and HT patients of the 1975-1982 series who were from the University of Pisa.

The selection criteria used for patients in the 5 disease groups in the 3 series were that no patient: 1) had to be treated with drugs, such as corticosteroids, capable of interfering with the immune system; 2) had received substitutive therapy with thyroid hormone or thyroid extracts; 3) had been treated with antithyroid drugs or ablative doses of ^{131}I or had undergone thyroidectomy. In GD, THAb are more frequent after, rather than before, antithyroid drug treatment (1). In addition, radioiodine and surgical manipulation of the thyroid may cause leakage of thyroid autoantigens that can favor synthesis of THAb (1). After our recent demonstration (6) that diagnostic fine-needle aspiration biopsy of the thyroid (FNAB) results in leakage of heterogeneous TG molecules which are indeed capable of eliciting THAb in patients who were THAb negative (THAb$^-$) prior to FNAB, from our 1998-1999 series we excluded patients who underwent FNAB prior to sampling. For sake of homogeneity, in the 1998-1999 series we did not enroll patients who underwent diagnostic puncture of the salivary glands, arthrocentesis or intra-articular injection of drugs. Clearly, these exclusion criteria obstacle, rather than favor, the findings we expected. Fourteen SS patients gave consent to perform a labial salivary gland biopsy, which was diagnostic. However, THAb were measured on blood that had been drawn at the time of the first visit, weeks before biopsy.

THAb were also measured in 70 adult patients with euthyroid non-autoimmune goiter who were sampled in 1998-1999.

Diagnosis of SS, RA and other collagenoses (OADCT)

No international uniform classification system for SS exists. We used the European Union classification criteria prepared and validated by an *ad hoc* committee of experts (7). The presence of

Table 1 - *Comparison of the 5 disease groups of patients collected in 3 distinct periods of time*.*

Parameter	Hashimoto's thyroiditis			Graves' disease			Sjögren syndrome**			Rheumatoid arthritis			Other collagenoses°		
	'75-'82	'90-'92	'98-'99	'75-'82	'90-'92	'98-'99	'75-'82	'90-'92	'98-'99	'75-'82	'90-'92	'98-'99	'75-'82	'90-'92	'98-'99
no.	239	75	88	163	31	25	5	13	20	7	16	23	6	16	20
F/M	226/13	69/6	77/11	133/30	26/5	20/5	5/0	12/1	19/1	5/2	12/4	18/5	6/0	13/3	17/3
Age (yr) m±SD	50±11	46±12	42±9	43±10	41±8	41±10	52±13	55±15	51±12	47±9	49±11	45±10	33±5	40±7	38±6
TgAb+°° no.	174	49	51	85	21	16	1	2	7	1	1	0	0	2	1
%	73%	65%	58%	52%	68%	64%	20%	15%	35%	14%	6%	0	0	12%	5%
TgAb+°° no.	216	61	64	116	26	20	2	5	10	1	4	6	1	4	3
%	90%	81%	73%	71%	84%	80%	40%	38%	50%	14%	25%	30%	17%	24%	15%

*The 5 disease groups of the 1975-1992 series were studied in a previous paper (3). The 163 Graves' disease patients of this chronological series includes the 154 cases mentioned there (3) plus 9 additional cases collected in 1982 but evaluated for thyroid hormone autoantibodies (THAb) after publication of the paper (3). Of these 9 patients, one had IgG T_3 THAb. **Note that our 1998-1999 series is similar to the Perez *et al.* (5) for the listed parameters. In fact, in the latter series (5), number is 33, F/M ratio 16/1, age is 50±13 yr, prevalence of TGAb$^+$ and TPOAb$^+$ is 18% and 45%, respectively. °In addition to the 6 patients with other collagenoses (all 6 with systemic lupus erythematosus, SLE), the following patients with lymphoreticular disorders were studied in our cited paper (3): 2 with Waldenström macroglobulinemia, 4 with plasmacytoma, 1 with heavy chain disease, 2 with double light chain disease, 2 with benign monoclonal gammopathy, 1 lymphocytic lymphoma. All these 18 patients tested THAb negative. The 16 patients with other collagenoses collected in the 1990-1992 included 10 patients with SLE, 4 with polymyositis and 2 with scleroderma. °°In the years 1975-1982, the method used for measuring TgAb and TPOAb was tanned red cells hemoagglutination (kit by Wellcome, Rome, Italy, and kit by Fujizoki, Tokyo, Japan, respectively). At that time, TPOAb were known as thyroid microsomial Ab. In the years 1990-1992, the 2 antibodies were measured with the RIA kits by Radim (Genova, Italy). In the years 1998-1999, TgAb were assayed with the immunoradiometric kit by CIS, TPOAb with the RIA kit by Sorin (TPOAb) (see text, Patients and methods section). TGAb: thyroglobulin antibody; TPOAb: thyroid peroxidase antibody.

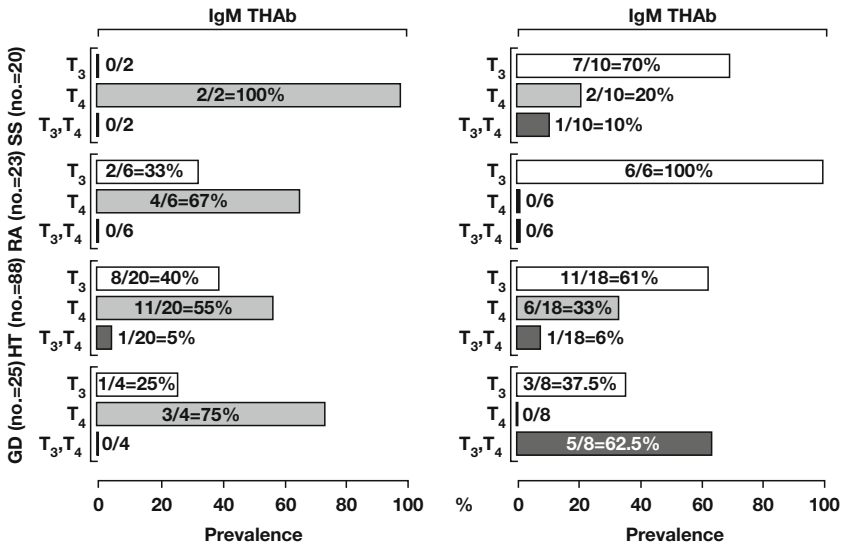

Fig. 1 - *Hormone specificity of thyroid hormone autoantibodies (THAb) of either the immunoglobulin M or G (IgM, IgG) class in 4 disease groups whose sera were collected in 1998-1999: primary Sjögren syndrome (SS), rheumatoid arthritis (RA), Hashimoto's thyroiditis (HT) and Graves' disease (GD). THAb were measured with the radioimmunoprecipitation method described previously (4, 6). Immunoprecipitated over total radioactivity added (% B/T; mean±SD) ranged 4.80 to 8.20% (T_3 IgM), 4.60 to 10.50% (T_4 IgM), 4.80 to 15.0% (T_3 IgG) and 5.10 to 11.70% (T_4 IgG).*

4 out of 6 items allows the definitive diagnosis of primary SS without the necessity of performing the salivary gland biopsy. The 6 items are: ocular symptoms, oral symptoms, ocular signs, histopathological features, salivary gland involvement and autoantibodies (positive anti-Ro/SS-A and anti-La/SS-B serum antibodies). We excluded associated collagenoses that, if present, are diagnostic of secondary SS, on the basis of the same "well-defined commonly accepted criteria (8-12) utilized by the European Community study" (7). Particularly, the criteria used to exclude RA (13) or SLE (14), scleroderma (8) and polymyositis (9) coincided with the criteria that our patients had to fulfill to enter the RA or the other collagenoses group, respectively.

The prevalence of pertinent autoantibodies in the 1975-1982, 1990-1992 and 1998-1999 series of patients were: 65%, 61% and 60% (SS-A Ab+SS-B Ab in primary SS patients), 74%, 75% and 71% (rheumatoid factor in RA patients), 100%, 100% and 100% (anti-nuclear Ab in SLE patients).

THAb assay

Each THAb was measured in duplicate with the radioimmunoprecipitation technique described previously in detail (4, 6), using anti-human IgM or anti-human IgG serum (Behring, Scoppito, Italy). In brief, 500 µl serum were incubated with 0.5 µCi [^{125}I]T_3 or [^{125}I]T_4 (NEN Life Science Products, Brussels, Belgium) for 60 min at 23 C. Twenty microliters of this mixture were then incubated with 150 µl anti-h-IgM or anti-h-IgG, both prediluted 1:10 with saline containing BSA (Sigma, St Louis, U.S.A.) at a final concentration of 0.5%. After 24 h incubation at 4 C, tubes were centrifuged at 2,000 x g for 20 min and the supernatant was aspirated. Sera were considered THAb$^+$ when the immuno-precipitated radioactivity (per cent bound over total added) was more than 2 standard deviations (SD) from the normal mean. These cut-off points were 3.9% (T_3IgM), 3.6% (T_3IgG), 3.4% (T_4IgM) and 3.9% (T_4IgG). When levels were above normal, and therefore positive, or borderline, THAb were reassayed twice. Although the inter-assay coefficients of variations for the 4 THAb assays are low (3.1 to 3.5%) (6), measurement of THAb was performed in 4 distinct assays: one for T_3IgM, one for T_3IgG, one for T_4 IgM and one for T_4 IgG. Any given assay included all patients of the 5 disease groups, and ignoring to which group patients belonged and whether there were already hints of the presence of THAb.

The suspicion for THAb, based on the known discrepancy between thyroid hormone levels and the functional status, existed for 4 SS patients (cases no. 6, 9-11), 4 RA patients (no. 7-10) and 10 HT patients (no. 14, 15, 23-30) who had already measured free thyroid hormones and TSH. Serum FT_3 and/or FT_4 had resulted spuriously high or normal, and inconsistent with their euthyroid or overtly hypothyroid status, in all these 18 patients.

Thyroid antibody assays

TGAb and thyroid peroxidase Ab (TPOAb) were measured with the immunoradiometric kit by CIS (Gif-sur-Yvette, France) and RIA kit by Sorin (Saluggia, Italy), respectively. The corresponding normal values are 0-50 and 0-10 U/ml.

Statistics

Data are given as mean±SD. Differences between means were evaluated by the two-tailed Student's *t* test. Differences between proportions were evaluated by the Fisher's exact test or the χ^2 test as appropriate. In all 3 tests, the level of statistical significance was set at p=0.05.

RESULTS

1998-1999 series: Prevalence of THAb

Overall prevalence of THAb (of either class) in primary SS, RA, other collagenoses, GD and HT was 55% (11/20), 40% (10/25), 0%, 36% (9/25) and 34% (30/88), respectively.

Prevalence based on class and hormone specificity is summarized in Figure 1, except for collagenoses other than SS and RA because THAb were always undetectable. Prevalence of IgM-THAb was not sta-

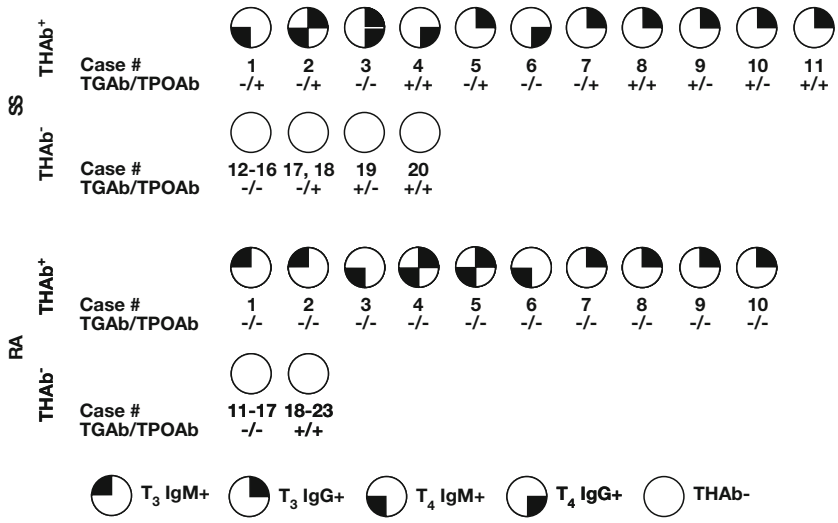

Fig. 2 - *Thyroid hormone autoantibodies (THAb) in patients with primary Sjögren syndrome (SS) or rheumatoid arthritis (RA), and their relation to thyroglobulin antibody (TGAb) and thyroid peroxidase antibody (TPOAb). Symbols for thyroid hormone specificity and class of the THAb are at the bottom. As in Figures 1, 3 and 4, patients are from the 1998-1999 series.*

tistically different (p>0.05) between groups: 26% (6/23) in RA, 23% (20/88) in HT, 16% (4/25) in GD and 10% (2/20) in SS. Prevalence of IgG-THAb was 50% (10/20) in SS, 32% (8/25) in GD, 26% (6/23) in RA and 20% (18/88) in HT, and only the comparison between SS and HT resulted statistically significant (χ^2=7.388; p<0.01).

Serum concentrations of IgM-THAb and IgG-THAb were not statistically different either within disease or between diseases (data not shown). THAb were undetectable in the sera of the 70 patients with non-autoimmune goiter, thus confirming previous data on a larger series (6).

1998-1999 series: Types of THAb

Regardless of disease, the most frequent IgM-THAb were directed against T_4, while the most frequent IgG-THAb were directed against T_3 (Fig. 1), the exception being GD (T_3 T_4 IgG >T_3 IgG). Regardless of disease, THAb of several types (based on class, specificity or both) may circulate in any given patient (Fig. 2, 3), but only in GD patients (cases no. 1 and 3) we could detect 3 types of THAb simultaneously.

Single types of THAb prevailed in SS (9/11 or 82%) and RA (8/10 or 80%) (Fig. 2); either rate was significantly greater than in GD (3/9 or 33%; p=0.037

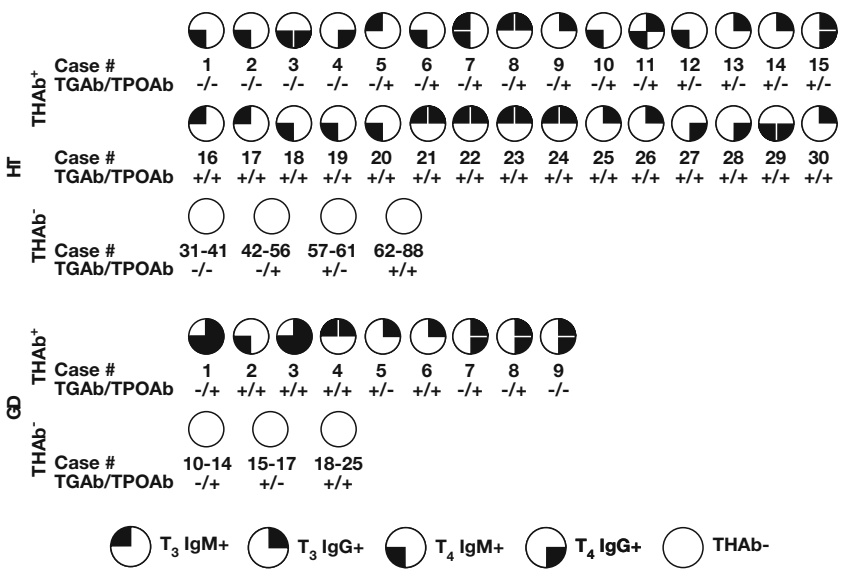

Fig. 3 - *Thyroid hormone autoantibodies (THAb) in patients with Hashimoto's thyroiditis (HT) or Graves' disease (GD), and their relation to TGAb and TPOAb (see legend of Fig. 2 for details). Symbols for THAb class and hormone specificity are given at the bottom.*

or p=0.05, respectively), but not in HT (20/30 or 67%) (Fig. 3). Mixed types of THAb concordant for specificity were never detected in SS or RA, and rarely in HT and GD, with no statistical difference between the 4 diseases. Particularly, T_3IgM+T_3 IgG accounted for 5/30 (17%) of the HT THAb and 1/9 (11%) of the GD THAb; T_4IgM+T_4IgG accounted for 2/30 (7%) and 2/9 (22%), respectively. Mixed types of THAb concordant for class (namely, T_3IgM+T_4 IgM, T_3IgG+T_4IgG) were never observed in RA; T_3 IgM+T_4 IgM were also absent in SS and GD.

Of the totally discordant THAb, T_3IgG+T_4 IgM were present in all groups but at relatively low rates (1/9 or 11% in SS, 2/10 or 20% in RA, 1/30 or 3% in HT, 2/9 or 22% in GD).

1998-1999 series: Relationship between THAb and TGAb

Thyroid-stored iodinated TG is responsible for eliciting THAb synthesis (6), but, as already mentioned in the Introduction, a not negligible proportion of $THAb^+$ subjects are $TGAb^-$. It was therefore of interest to examine the relationship between THAb and TGAb in our patients, and this is shown in Figure 2 for SS and RA, and Figure 3 for HT and GD. The proportion of undetectable TGAb in $THAb^+$ patients were 100% (10/10), 55% (6/11), 44% (4/9) and 37% (11/30) in RA, SS, GD and TH, respectively. Only the differences between RA and each of the other 3 diseases were statistically significant (p=0.035 to 0.005).

The risk of having THAb in patients categorized based on the TGAb status can be appreciated in Figure 4. Approximately, one out of 3 HT patients develop THAb regardless of the TGAb status. In GD the risk of THAb is approximately one third for $TGAb^+$ patients, not statistically different from the half risk for $TGAb^-$ patients (p=0.27). The approximate half risk of developing THAb for the $TGAb^-$ status also exists in primary SS and RA patients. The highest risk of THAb, approximately three quarters, is in $TGAb^+$ primary SS patients (p=0.08 *vs* GD, p=0.07 *vs* HT).

Comparison between the 3 chronological series: Prevalences of THAb

This comparison, possible as the same methodology and antisera from the same commercial source was always used, is illustrated in Figure 5. Data are presented for the IgG-THAb, because only the IgG class was studied in the 2 earliest series. Regardless of disease, there is an unequivocal increase in prevalence over the years. Compared to about 20-25 yr ago, the largest increases were observed for RA (>26-fold), HT (20-fold) and GD (16-fold). Prevalence of IgG-THAb in GD were slightly higher than in HT, and constantly so (1.6- to 2.2-fold more).

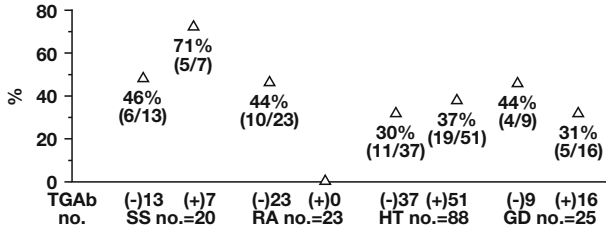

Fig. 4 - *Risk for developing thyroid hormone autoantibodies (THAb) in patients with primary Sjögren syndrome (SS), rheumatoid arthritis (RA), Hashimoto's thyroiditis (HT) and Graves' disease (GD) based on concurrent negative (-) or positive (+) status for circulating TG autoantibodies (TGAb).*

THAb predictivity for subsequent autoimmune hypothyroidism

Because progression of autoimmune thyroiditis to thyroid failure is slow (10), the possibility that the presence of serum THAb may precede (and therefore be predictive of) hypothyroidism could be evaluated only in the 1975–1980 and 1990–1992 series. Concerning SS, the only TPOAb/TGAb/$THAb^+$ patients of the first series and two quarters similar patients of the second series developed subclinical or overt hypothyroidism within the following 10 yr. TGAb continued to be undetectable but TPOAb had converted to positive when hypothyroidism was diagnosed. Concerning RA, half $TPOAb^-$/$TGAb^-$/$THAb^+$ patients of the second series became subclinically hypothyroid within the following 10 yr, and both TGAb and TPOAb converted to positive at that time.

$TGAb^-$/$THAb^+$ was one third HT patient of the

Fig. 5 - *Prevalence of immunoglobulin G-thyroid hormone autoantibodies (IgG-THAb) in 4 disease groups of patients whose blood was collected for measuring serum THAb in 3 distinct periods of time: years 1975-1982, 1990-1992 and 1998-1999. GD: Graves' disease; HT: Hashimoto's thyroiditis; RA: rheumatoid arthritis; SS: primary Sjögren syndrome.*

1990–1992 series, but she died at the 4th yr of follow-up while still euthyroid.

DISCUSSION

In the present study we have shown the high frequency of serum THAb in primary SS and RA, which is even greater than that of classical antithyroid antibodies (TGAb, TPOAb). While the high prevalence of THAb in primary SS was previously reported (4, 5), data for RA are novel. Moreover, we report that the frequency of THAb in primary SS, RA, HT and GD has increased steadily over the last 25 yr, which is impressive considering that, in the 1998-1999 series, we excluded patients with conditions that favor appearance of THAb. This increase is also apparent when the 2 extremes of the past decade are compared, and it is worthy of note that it was of similar magnitude for similar diseases, *viz.*, 5-fold in HT and GD, and ≤2-fold in SS and RA. Hence, it will be of interest to evaluate whether the trend continues in the following decades.

The high prevalence of THAb in SS and RA cannot be due to the autoimmune background *per se*, because in other collagenoses and in various disorders of the lymphoreticular system, THAb are consistently absent [(4) and present study]. To the best of our knowledge, only one case of SLE-associated THAb is reported in the literature (11), but the patient had concurrent, secondary SS. Based on the bulk of our data, we should conclude that SS, not SLE, predisposed this Japanese woman to develop THAb. The THAb of this patient (11) were T_3IgG, a data which matches our finding of T_3IgG being the most frequent THAb in primary SS.

As thyroid hormones are synthesized solely in the thyroid, how can the chronic autoimmune inflammation of extra-thyroidal tissues elicit THAb, and with a frequency greater than in the chronic autoimmune inflammation of the thyroid? There are a number of possibilities, the illustration of which requires the mentioning of 4 key-data. First, exposure of hidden autoantigens and/or TG-like epitopes is facilitated by the tissue damage caused by locally released cytokines (3, 10, 12, 15). Second, lymphocytic infiltration of variable degree is present in several organs of patients with either RA or SS (16), and thyroid infiltration can well cause sufficient damage to allow exposure of iodinated epitopes of TG and/or leakage of iodinated and immunogenic TG fragments. Third, the lymphocytic infiltrate associated to HT, RA and SS consists of both T and B lymphocytes (3, 9, 15), the latter synthesizing autoAb against local tissue proteins. Fourth, since post-translational modifications of TG (17) can enable serum TGAb to cross-react with similarly glycosylated or phosphorylated or sulfated proteins of tissues other than thyroid (18), the reverse can be true – namely, autoAb may circulate, directed against autoantigens of joints or exocrine glands, which share chemical similarity with iodinated regions of TG. At least in the case of RA, such chemical similarity might concern the chondroitin sulfate unit of TG (17), as chondroitin sulfates are prominent components of the cartilage (19).

Thus, one answer to the above question is that B lymphocytes of RA and SS patients would produce Ab against one or more connective proteins that share(s) chemical or amino acid sequence similarity with iodinate regions of TG. These Ab will therefore react with thyroid hormones. Indeed, the early stages of lymphocytic thyroiditis are characterized by serum reactivity against either thyroid hormone even tough TGAb that can be assayed by conventional methods are undetectable (18). As the inflammatory process in the thyroid grows and more thyroid is damaged (which is the case of HT), more thyroid autoantigens are exposed and released into the general circulation, thus increasing activation of more lymphocytes with different specificities. The end result is an overall diversity of the TGAb response; TGAb have become more polyclonal, and hormonogenic domains are no longer immunodominant (18). The combined effect of this lack of switch in TGAb specificity and the mechanism of molecular mimicry, could explain why prevalence of THAb is in primary SS and RA greater than in autoimmune thyroid diseases.

One other possibility is that, from the damaged extra-thyroid tissues of RA and SS, some material leaks which is capable of binding thyroid hormones, thus forming novel iodinated autoantigens. These new autoantigens need to be antigenically more potent, in terms of eliciting THAb, than iodinated TG in order to explain the greater prevalence of THAb in SS and RA. Because there is no TG involvement, this can explain the association of THAb with the absence of circulating TGAb.

Environmental factors, such as stress, smoke, toxins, pollutants, bacteria or viruses, play a contributing or triggering role in the pathogenesis of HT, SS and RA (13, 20-23). Perhaps, THAb are becoming more frequent, regardless of disease, because of a greater exposure of predisposed individuals to these exogenous factors. At least in HT, the increasing prevalence of THAb is only one of the sev-

eral modifications we have noted over the last 25 years (24).

Our findings have also practical implications. For instance, one could target SS and RA patients who are to be followed up for subsequent development of HT. If TGAb and TPOAb are used for such screening, then one misses patients who are $TGAb^-/TPOAb^-$ but $THAb^+$. As mentioned in the Results, THAb may anticipate by years the appearance of TGAb, TPOAb and hypothyroidism.

Other practical implications are issues of misdiagnosis and mistreatment resulting from the interference of THAb on thyroid hormone assays, very often an overestimation (1), as illustrated by 2 of the 18 patients in the 1998-1999 series mentioned under Patients and Methods. The spurious overestimation of serum FT_4 in one $TGAb^-/TPOAb^-/THAb^+$ SS patient led one endocrinologist to exclude the possibility of early hypothyroidism *a priori*, because both FT_4 and TSH were in the upper-normal range. However, TSH response to iv TRH (baseline=4.0, peak=31.8 mU/l) proved that the patient was subclinically hypothyroid. In the second patient, a woman suffering from RA with T_3 IgG who had symptoms suggestive of hyperthyroidism and GD had been diagnosed based on borderline levels of TPOAb, subnormal TSH (while on corticosteroids!) and spuriously high FT_3. Methimazole was inappropriately given, and unnecessary side-effects appeared.

In conclusion, TPOAb and TGAb cannot any longer be considered the sole serum markers of concurrent thyroid autoimmunity in patients with primary SS and RA, and indeed their prevalence is equal to and lower than THAb, respectively. In both SS and RA patients who are $TGAb^-$, detection of THAb can be predictive of subsequent HT. Special studies are required to explain the peculiar association of THAb with these 2, but not other, collagenoses and to identify the possible antigen(s) which share(s) sequence and/or chemical homology with TG.

REFERENCES

1. Benvenga S., Trimarchi F., Robbins J. Circulating thyroid autoantibodies. J. Endocrinol. Invest. 1987, *10:* 605-619.
2. Elsing C., Pilar K., Hehrmann R. Haufgeikt und Klinische Bedeutung Zirkkiliender Autoantikorper gegen Schildrussenhormone. Akt. Endokr. Stoffw. 1991, *12:* 49-56.
3. Larsen P.R., Davies T.F., Hay I.D. The thyroid gland. In: Wilson J.D., Foster D.W., Kronenberg H.M., Larsen P.R. (Eds.), Williams Textbook of Endocrinology. W.B. Saunders, Philadelphia, 1998, p. 389.
4. Trimarchi F., Benvenga S., Costante G., *et al*. Identification and characterization of circulating thyroid hormone autoantibodies in thyroid diseases, in autoimmune non-thyroid illnesses and in lymphoreticular disorders. J. Endocrinol. Invest. 1983, *6:* 203-209.
5. Perez E.B., Kraus A., Lopez G., Cifuentes M., Alarcon-Segovia D. Autoimmune thyroid diseases in primary Sjögren syndrome. Am. J. Med. 1995, *99:* 480-484.
6. Benvenga S., Bartolone L., Squadrito S., Trimarchi F. Thyroid hormone autoantibodies elicited by diagnostic fine-needle biopsy. J. Clin. Endocrinol. Metab. 1997, *82:* 4217-4223.
7. Vitali C., Bombardieri S., Moutsopoulos H.M., *et al*. for the European Community Study Group on the Diagnostic Criteria for Sjögren sindrome. Preliminary criteria for the classification of Sjögren syndrome. Results of a prospective concerted action supported by the European Community. Arthritis Rheum. 1993, *36:* 340-347.
8. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum. 1980, *23:* 581-590.
9. Bohan A., Peter J.B. Polymiositis and dermatomyiositis. Part I and part II. N. Engl. J. Med. 1975, *292:* 344-403.
10. Weetman A.P. Chronic autoimmune thyroiditis. In: Bravermann L.E., Utiger R.D. (Eds.), Werner and Ingbar's The Thyroid. Lippincott-Raven, Philadelphia, 1996, p. 738.
11. Komaki T., Sakata S., Nakamura S., Kamiko K., Miura K. Two cases associated with thyroid hormone autoantibodies. In: Walfish P.G., Wall J.R., Volpe R. (Eds.), Autoimmunity and the thyroid. Academic Press, Orlando, 1985, p. 403.
12. Lipsky P.E. Artrite reumatoide. In: Isselbacher K.J., Braunwald E., Wilson J.D., Martin J.B., Fauci A.S., Kasper D.L. (Eds.), Harrison Principi di Medicina Interna. McGraw-Hill, Milan, 1995, p. 1868.
13. Arnett F.C., Edworthy S.M., Bloch D.A., *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988, *31:* 315-324.
14. Tan E.M., Cohen A.S., Fries J.F., *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1982, *25:* 1271-1277.
15. Moutsopoulos H.M. Sindrome di Sjögren. In: Isselbacher K.J., Braunwald E., Wilson J.D., Martin J.B., Fauci A.S., Kasper D.L. (Eds.), Harrison Principi di Medicina Interna. McGraw-Hill, Milan, 1995, p. 1885.
16. Fye K.H., Sack K.E. Rheumatic diseases. In: Stites D.P., Stobo J.D., Fudenberg H.H., Wells J.V. (Eds.), Basic and Clinical Immunology. Lange Medical Publications, Los Altos, 1982, p. 423.
17. Dunn J.T., Dunn A.D. Thyroglobulin: chemistry, biosynthesis and proteolysis. In: Bravermann L.E., Utiger R.D.

(Eds.), Werner and Ingbar's The Thyroid. Lippincott-Raven, Philadelphia, 1996, p. 90.

18. Kuppers R.C., Bresler H.S., Burek C.L., Gleason S.L., Rose N.R. Immunodominant determinants of thyroglobulin-associated with autoimmune thyroiditis. In: Bona C.A., Kaushick A.K. (Eds.), Molecular immunobiology of self-reactivity. Marcel Dekker, New York, 1992, p. 247.
19. Martin D.W. Jr. Glycoproteins, proteoglycans and glycosaminoglycans. In: Martin D.W. Jr, Mayes P.A., Rodwell D.W., Granner D.K. (Eds.), Harper's review of Biochemistry. Lange Medical Publications, Los Altos, 1985, p. 464.
20. Brucker-Davis F. Effects of environmental synthetic chemicals on thyroid function. Thyroid 1998, *8:* 827-856.
21. Rasmussen A.K., Hartoft-Nielsen M.L., Feldt-Rasmussen U. Models to study the pathogenesis of thyroid autoimmunity. Biochimie 1999, *81:* 511-515.
22. Rhodus N.L. Sjögren's syndrome. Quintessence Intl. 1999, *30:* 689-699.
23. Danieli G., Gabrielli A., Pomponio G. Etiologia e patogenesi dell'artrite reumatoide. In: Dammacco F., Danieli G. (Eds.), Clinica Immunologica. Il Pensiero Scientifico, Roma, 1997, p. 189.
24. Benvenga S., Ruggeri R.M., Lapa D., Russo A., Campenni A., Trimarchi F. The current presentation of Hashimoto's thyroiditis is different from that of the 1970's. 12th International Thyroid Congress, Kyoto (Japan), October 22-27, 2000. Endocr. J. 2000, *47* (Abstract: 192 P-345/D) (Suppl.): 192.