

Humoral Autoimmune Manifestation in Subacute Thyroiditis

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To study the autoimmune manifestations in subacute thyroiditis (SAT), the patterns of thyroid antibodies, thyroglobulin and circulating immune complexes were investigated in 10 patients during the course of the disease. Eight patients were thyrotoxic at diagnosis, and became euthyroid during recovery with a median observation of 8 months (4-30 months). Thyroid stimulating immunoglobulins were measured as TSH binding inhibiting immunoglobulins (TBII) and as thyroid stimulating antibodies (TSAb). TBII were present in all patients at least once during the observation period and remained detectable in six patients after recovery. TSAb were detected in three patients without relation to the hyperthyroid state. Thyroglobulin antibodies (TgAb) were present in four patients and persisted in three, while microsomal antibodies (MAb) were negative. Thyroglobulin (Tg) in the TgAb negative patients ($n = 6$) was high at diagnosis (median 229 µg/l, range 55-375) and fell rapidly during the course of SAT. Circulating immune complexes (CIC), which were found in all patients, reached maximal levels shortly after the onset of the disease and persisted after recovery. No correlation could be demonstrated between the different thyroid antibodies, and there was no clear relation between the levels of CIC and presence of the autoantibodies. However, the changes in CIC paralleled the changes in TBII, and it is suggested that immune complex formation is a major feature of the regulatory mechanisms controlling the immune responses in SAT.

Key words: circulating immune complexes; subacute thyroiditis; thyroglobulin; thyroid antibodies; thyroid stimulating immunoglobulins.

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Autoimmune thyroiditis can be classified into chronic progressive forms: Hashimoto's thyroiditis and Graves' disease, and the reversible forms: subacute thyroiditis (SAT) and silent thyroiditis (5). The aetiology of SAT is unknown, but in contrast to chronic autoimmune thyroiditis a viral pathogenesis has been evidenced (31), and both the immune mechanisms and the genetic predisposition are different (1, 28). It has been claimed that thyroid autoantibodies, present in most patients with

chronic autoimmune thyroiditis, are seldom in SAT, and although transitory autoimmune manifestations have been demonstrated, they are regarded as secondary to inflammatory release of antigen (31). However, recent studies might suggest that thyroid autoantibodies occur more frequently than previously assumed (32). Thus, the aim of the present investigation was to study the patterns of thyroid antibodies and circulating immune complexes (CIC) in relation to the course and recovery of SAT.

MATERIAL AND METHODS

Patients

The material comprised 10 consecutive patients (4 men and 6 women) with a mean age of 48 ± 12 years (SD). The diagnosis of SAT was based on the acute onset of symptoms of neck pain, fever, increased sedimentation rate, diffuse goitre, no uptake by ^{99m}Tc scintigraphy or by 24-h radioactive iodine uptake of the thyroid gland, and the later spontaneous recovery of thyroid function. Six patients were treated with steroids during the acute phase of SAT and all received analgetics. Six patients were studied from diagnosis (the acute phase) and all were followed until recovery (the recovery phase, median 6 months, range 4 to 30 months).

Laboratory methods

Total serum thyroxine (T_4), triiodothyronine (T_3) and thyrotropin (TSH) were measured by radioimmunoassays using routine methods, and the free indices (FI) of T_4 and T_3 were calculated by multiplication with the T_3 uptake test.

Serum thyroglobulin (Tg) was measured by a modification of a double antibody radioimmunoassay (17) using Tg and ^{125}I -Tg prepared as previously described (15). The total assay precision during 1 year was 11 % ($n = 40$) and the within assay precision 8 %. The 95 % reference interval based on 67 persons was 6.8–44 $\mu\text{g/l}$ (median 17.9 $\mu\text{g/l}$). Serum anti-thyroglobulin antibodies (TgAb) were measured by a radiocoprecipitation assay (12) using Medical Research Council Standard A 65/93 which by definition contained 1 Mega unit per litre (MU/l). The total assay precision in the concentration range around 1 MU/l was 7–12 %, whereas the within assay precision was 1.9–3.5 %. The 97.5 percentile (upper reference limit) of 163 controls was 0.028 MU/l.

Antimicrosomal antibodies (MAb) were detected by immunofluorescence technique (33).

Thyroid stimulating immunoglobulins were measured by two different methods: a) The radioreceptor assay for TSH binding inhibiting

immunoglobulins (TBII) as described by Bliddal (6) and calculated as a percentage inhibition of ^{125}I -TSH binding by the patient-IgG compared with a normal reference. The reference IgG was prepared from a pool of sera from 20 healthy donors without family history of thyroid disorders and was included in eight separate incubations in each assay. The reference range (97.5 percentile of 52 controls) was below 25 %. The within assay precision was 4 % and the total precision 10 % (7). b) The adenylate cyclase stimulation assay, as described by Bech & Madsen (2), measured thyroid stimulating antibodies (TSAb) as a percentage stimulation by the patient-IgG compared with a normal reference of 90 healthy donors. The reference range (95 percentile of 72 controls) was 71–110 %, the within assay precision 3 % and the total assay precision 11.5 % (7). In both assays IgGs from the references were included in eight separate incubations together with known normal and toxic IgGs as controls.

CIC were measured by a complement consumption assay after precipitation with 2.5 % polyethylene glycol (PEG-CIC) (9). Tetanus toxoid anti-tetanus toxoid complexes were used as standards in the complement consumption step, and the levels of CIC were expressed in terms of tetanus toxoid (TT) ($\mu\text{g/ml}$) in equivalent amount of standard complexes. The detection limit was 1.1 $\mu\text{g TT/ml}$.

In all analyses samples from one patient were run in the same assay to avoid interassay variation. All samples were measured in triplicate.

Statistical methods

To test significance the Mann-Whitney U-test (two-tailed) and Spearman rank correlation (one-tailed) were used with a significance level of 0.05.

RESULTS

All patients except two had elevated FT_4I at diagnosis (mean 15.9 ± 6.8 , normal range 5.0–11.0). The mean levels normalized rapidly within 2 months (Fig. 1) through a short phase

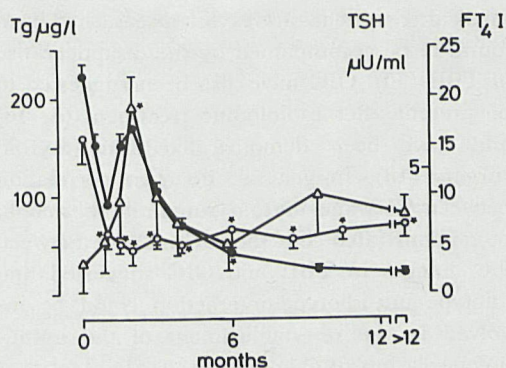


Fig. 1. The mean levels ($\pm$ SEM) of thyroglobulin (Tg) μ g/l ($n = 6$, $\bullet$), free thyroxine index (FT_4I) ($n = 10$, $\circ$), and thyrotrophin (TSH) μ U/ml ($n = 10$, Δ) during the course of subacute thyroiditis. The * indicate $2 P < 0.01$ (Mann-Whitney U-test) when compared with values at diagnosis (0 months).

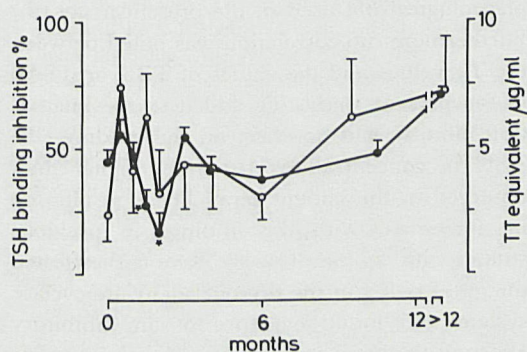


Fig. 2. The mean level ($\pm$ SEM) of the binding inhibiting immunoglobulins (TBII) measured by radioreceptor assay and expressed as percentage of inhibition ($n = 10$, $\bullet$), and circulating immune complexes measured by the complement consumption assay after PEG precipitation and expressed as tetanus toxoid (TT) μ g/ml in equivalent amounts of standard tetanus toxoid anti-tetanus toxoid complexes used ($\circ$). The * indicate $2 P < 0.01$ (Mann-Whitney U-test) when compared with the value at diagnosis (0 months).

of hypothyroidism evidenced by a significant increase in TSH ($P < 0.01$) (Fig. 1). A similar pattern was found when measuring serum Tg in six patients negative for TgAb. At diagnosis, Tg was high, but fell significantly parallel to TSH and FT_4I , until after 1 month when a secondary peak was recorded at 2 months simultaneously with the highest TSH concentration. After recovery, normal values were seen again (Fig. 1, $P < 0.01$). Thyroid stimulating immunoglobulins measured as TBII

were present in four of six patients tested at diagnosis, but all patients became positive during the disease period with a significant intermediate fall (Fig. 2, $P < 0.01$). In four patients TBII disappeared again after recovery (4–9 months) while the levels increased in the rest. When stimulating immunoglobulins were measured as TSAb, no patients were positive at diagnosis, but three became positive at low levels during the disease. The stimulating activity did not coincide with the hyperthyroid state (Table 1). MAb were not detected in any of the patients, while TgAb at diagnosis were present in two of six patients tested and became detectable in a further two patients during the disease period (Table 1). TgAb disappeared in two patients, while the concentrations increased in the remaining two patients after recovery.

PEG-CIC were detected in all patients. The concentrations were lowest at diagnosis (Fig. 2), but increased rapidly within 1 week to high levels similar to the TBII changes. Although the changes were not significant, the levels were still high after recovery (Fig. 2). There was no correlation between TgAb and TSAb or TBII, and the TSAb and TBII levels did not correlate with Tg.

To test the possibility of methodological interference, ^{125}I -Tg was coprecipitated with the immunoglobulins by ammonium sulphate with a recovery of 0.67 ± 0.03 (SD) (60,000 cpm added, $n = 4$). In the receptor assay no significant effect was found by adding Tg in concentrations up to 10^3 ng/ml.

DISCUSSION

In the present study hyperthyroidism was evident in the majority of patients in the acute

Table 1

Presence of thyroid antibodies during the course of subacute thyroiditis

Phase	Acute	Intermediary	Recovery
TgAb	2/6	4/10	2/10
TSAb	0/6	3/10	3/10
TBII	4/6	8/10	6/10
MAb	0/6	0/10	0/10

phase of SAT. The thyrotoxicosis and the coincident high levels of Tg were due to cellular damage (20), evidenced by the low radioiodine uptake. Recently, anti-TSH-receptor antibodies have been demonstrated transiently in SAT, but without correlation to the activity of the disease (26, 27, 32, 34). Thyroid stimulating antibodies have not previously been demonstrated (27, 32). In the present study, TBII were detected in all patients with SAT, while, in accordance with others (26, 34), low levels of TSAb were found only in a minor part, without relation to the hyperthyroid episodes, and none showed MAb. Histological examination of the thyroid was not performed in our study, but the scintigrams indicated that the lesions were diffuse in the whole gland in all cases, in contrast to a recent report by Lio et al. (24) which also included patients with localized lesions. There have been several reports of transient hyperthyroidism in patients with lymphocytic thyroiditis and low thyroid radioiodine uptake (13, 21, 22). It is not clear whether these variants constitute a broad spectrum of a common disease or separate disease entities.

Experimental autoimmune thyroiditis (EAT) has served as a model for autoimmune diseases in general and produced a number of results on the interactions of antigen and antibody in the immune response. The lesions in EAT are unspecific and they disappear when the antigenic challenge is terminated. EAT can only be produced in normal animals and seems to depend on intact T-cells (25). There are several characteristic features of EAT which resemble SAT, and it has been suggested that both have similar development mechanisms. Thus, transient autoimmune manifestations have been demonstrated in SAT, such as presence of thyroid antibodies (31) and cellular immunity to thyroid antigens (29). It has been proposed that SAT is a self-limiting disorder because the regulatory mechanisms, including intact suppressor cell function controlling the immune responses to thyroid antigens, are probably normal (10, 23, 31). In the present study CIC could be detected in all patients, although they were not correlated to the patterns of antibodies. The occurrence of CIC in the course of

medical treatment of Graves' disease has been found to be accompanied by the disappearance of TBII (30). CIC have also been suggested to be present after radioiodine treatment (4, 16) and have been demonstrated after thyroid surgery (18). In general, no clear correlation between CIC and thyroid autoantibodies could be demonstrated, but the resemblance between the changes in TBII and CIC suggested that idiotype-anti-idiotype interaction could be involved in the re-establishment of the immunological control (14).

It has been suggested that Tg may interfere in the receptor assay in cases with high Tg levels (19). However, the binding of Tg to thyroid membranes seemed predominantly to take place at lower pH values (11) than the physiological pH used in the present assay (6). Furthermore, no correlation was noted between the Tg values and the values of TBII or TSAb in the present study. Tg added to the incubation mixture had no effect on the binding ^{125} I-TSH in concentrations 10^3 times higher than observed in the patient sera. These results are in agreement with the findings in previous studies with Tg (32, 34). We have earlier tested the effect of Tg in the thyroid adenylate cyclase system and found evidence of an inhibitory effect on the basal and stimulated activities, but no evidence of interference in the assay for TSAb (3). In conclusion, autoimmune manifestations are present in SAT for a rather long period. Thyroid-stimulating immunoglobulins are probably heterogeneous, but the relationships to CIC are unknown. Formation of immune complexes might be a major regulatory mechanism of the immune response in SAT, but further studies are needed to identify the components of CIC and the nature of these antibodies.

Four patients were tested for presence of CIC at 3, 3, 6 and 12 years, respectively, after the diagnosis of SAT, and all were negative.

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REFERENCES

1. Bech, K., Nerup, J., Thomsen, M., et al.: Subacute thyroiditis de Quervain: A disease associated with a HLA-B antigen. *Acta Endocrinol. (Kbh.)* 86, 504-509, 1977.
2. Bech, K. & Madsen, S. N.: Thyroid adenylate cyclase stimulating immunoglobulins in thyroid diseases. *Clin. Endocrinol. (Oxf.)* 11, 47-58, 1979.
3. Bech, K., Feldt-Rasmussen, U. & Madsen, S. N.: Influence of thyroglobulin on basal and stimulated human thyroid adenylate cyclase activity. *J. Clin. Endocrinol. Metab.* 53, 264-269, 1981.
4. Bech, K.: Immunological aspects of Graves' disease and importance of thyroid stimulating immunoglobulins. *Acta Endocrinol. (Kbh.)* 103, Suppl. 254, 1-40, 1983.
5. Bech, K., Bliddal, H., Feldt-Rasmussen, U., Kappelgaard, E. & Nielsen, H.: Heterogeneity of autoimmune thyroiditis. *Allergy* 39, 239-247, 1984.
6. Bliddal, H.: A stable, reproducible radioreceptor assay for thyrotrophin binding inhibiting immunoglobulins (TBII). *Scand. J. Clin. Invest.* 41, 441-450, 1981.
7. Bliddal, H., Bech, K., Petersen, P. H., Siersbæk-Nielsen, K. & Friis, T.: Evidence of a correlation between thyrotrophin receptor binding inhibition and thyroid adenylate cyclase activation by immunoglobulins in Graves' disease before and during long-term antithyroid treatment. *Acta Endocrinol. (Kbh.)* 101, 35-40, 1982.
8. Brown, R. S., Pohl, S. L., Jackson, I. M. D. & Reichlin, S.: Do thyroid-stimulating immunoglobulins cause non-toxic and toxic multinodular goitre? *Lancet* 1, 904-906, 1978.
9. Brandslund, I., Siersted, H. C., Jensenius, J. C. & Svehag, S. E.: Detection and quantitation of immune complexes with a rapid polyethylene glycol precipitation complement consumption method (PEG-CC). *Methods Enzymol.* 74, 551-571, 1981.
10. Chartier, B., Bandy, P. & Wall, J. R.: Fc Receptor-bearing blood mononuclear cells in thyroid disorders: Increased levels in patients with subacute thyroiditis. *J. Clin. Endocrinol. Metab.* 51, 1014-1018, 1980.
11. Consiglio, E., Salvatore, G., Rall, J. E. & Krohn, L. D.: Thyroglobulin interactions with thyroid plasma membranes. *J. Biol. Chem.* 254, 5065-5076, 1979.
12. Date, J., Feldt-Rasmussen, U., Petersen, P. H. & Bech, K.: An improved coprecipitation assay for determination of thyroglobulin antibodies. *Scand. J. Clin. Lab. Invest.* 40, 37-44, 1980.
13. Dorfman, S. G., Cooperman, M. T., Nelson, R. L., Depuy, H., Peake, R. L. & Young, R. L.: Painless thyroiditis and transient hyperthyroidism without goiter. *Ann. Intern. Med.* 86, 24-28, 1977.
14. Farid, N. R., Briones-Urbina, R. & Bear, J. C.: Graves' disease - The thyroid stimulating antibody and immunological networks. *Mol. Aspects Med.* 6, 355-457, 1983.
15. Feldt-Rasmussen, U., Petersen, P. H. & Date, J.: Sex and age correlated reference values of serum thyroglobulin measured by a modified radioimmunoassay. *Acta Endocrinol. (Kbh.)* 90, 440-450, 1979.
16. Feldt-Rasmussen, U.: Serum thyroglobulin and thyroglobulin autoantibodies in thyroid diseases. *Allergy* 38, 369-387, 1983.
17. Feldt-Rasmussen, U., Holten, I. & Sand Hansen, H.: Influence of thyroid substitution therapy and thyroid autoantibodies on the value of serum thyroglobulin in recurring thyroid cancer. *Cancer* 51, 2240-2244, 1983.
18. Feldt-Rasmussen, U., Hoier-Madsen, M., Date, J., Kappelgaard, E. & Husby, S.: Methodological aspects of thyroglobulin autoantibodies in serum and their relation to serum thyroglobulin. In Doniach, D., Schleusener, H. & Weinheimer, B. (eds.): Current topics on autoimmunity, pp. 101-109. Georg Thieme Verlag, Stuttgart, 1985.
19. Fenzi, G. F., Bartalena, L., Macchia, F., Monzani, F. & Checchi, M.: Interference of thyroglobulin in TSAb detection by radioreceptor assay. *J. Endocrinol. Invest.* 2, 221-222, 1979.
20. Greene, J. N.: Subacute thyroiditis. *Am. J. Med.* 51, 97-108, 1971.
21. Ginsberg, J. & Walfish, P. G.: Post-partum transient thyrotoxicosis with painless thyroiditis. *Lancet* 1, 1125-1128, 1977.
22. Gorman, C. A., Duick, D. S., Woolner, L. B. & Wahner, H. W.: Transient hyperthyroidism in patients with lymphocytic thyroiditis. *Mayo Clin. Proc.* 53, 359-365, 1978.
23. Iwatani, Y., Amino, N., Mori, H., et al.: T lymphocyte subsets in autoimmune thyroid diseases and subacute thyroiditis detected with monoclonal-antibodies. *J. Clin. Endocrinol. Metab.* 56, 251-254, 1982.
24. Lio, S., Pontecorvi, A., Caruso, M., Monaco, F. & D'Armiento, M.: Transitory subclinical and permanent hypothyroidism in the course of subacute thyroiditis (de Quervain). *Acta Endocrinol. (Kbh.)* 106, 67-70, 1984.
25. Rose, N. R., Kong, Y. M., Okayasu, I., Giraldo, A. A., Beisel, K. & Sundick, R. S.: T-cell regulation in autoimmune thyroiditis. *Immunol. Rev.* 55, 299-314, 1981.
26. Strakosch, C. R., Joyner, D. & Wall, J. R.: Thyroid stimulating antibodies in patients with subacute thyroiditis. *J. Clin. Endocrinol. Metab.* 46, 345-348, 1978.
27. Sugeno, A., Kidd, A., Row, V. V. & Volpé, R.: Correlation between thyrotropin-displacing activity and human thyroid-stimulating activity by immunoglobulins from patients with Graves' disease and other thyroid disorders. *J. Clin. Endocrinol. Metab.* 48, 398-402, 1979.
28. Thomsen, M., Ryder, L. P., Bech, K., et al.: HLA-D in Hashimoto's thyroiditis. *Tissue Antigens* 21, 173-175, 1983.
29. Tötterman, T.: Distribution of T- and B-, and thyroglobulin-binding lymphocytes infiltrating the gland in Graves' disease, Hashimoto's thyroiditis and de Quervain's thyroiditis. *Clin. Immunol. Immunopathol.* 10,

- 270-277, 1978.
30. Van der Heide, D., De Bruin, J. H., van Es, T. W. A., et al.: Circulating immune complexes and thyroid stimulating immunoglobulins before, during and after anti-thyroid drug therapy in patients with Graves' disease. *Lancet* *1*, 1376-1379, 1980.
31. Volpé, R.: Subacute de Quervain's thyroiditis. *Clin. Endocrinol. Metab.* *8*, 81-95, 1979.
32. Wall, J. R., Strakosch, C. R., Bandy, P. & Bayly, R.: Nature of thyrotropin displacement activity in subacute thyroiditis. *J. Clin. Endocrinol. Metab.* *54*, 349-353, 1982.
33. Weller, T. H. & Coons, A. H.: Fluorescent antibody studies with agent of varicella and herpes propagated in vitro. *Proc. Soc. Exp. Biol.* *86*, 789-794, 1954.
34. Wägar, G. & Mäkinen, T.: Thyrotropin-binding inhibiting immunoglobulins (TBII) in subacute thyroiditis. *Acta Endocrinol. (Kbh.)* *102*, Suppl. 251, 53-57, 1983.

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