

In vitro Characterization of Synthesized Thyroglobulin Immune Complexes (IC)

A Model for Studies of IC Containing an Autoantigen

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Human thyroglobulin (Tg) immune complexes (TgIC) were preformed at different ratios from purified Tg, and Tg antibodies (TgAb) obtained from six patients with autoimmune thyroid diseases. TgIC were characterized by precipitation with polyethylene glycol, and sedimentation in sucrose gradient. TgIC from one patient were further characterized by concanavalin A (con A), and binding to the complement factor Clq and rheumatoid factor (RF).

Tg and TgIC had almost identical binding to con A, no binding to Clq, but could be partially separated by ultracentrifugation and polyethylene glycol. The highest degree of separation was obtained by a RF-coated plastic tube radiometric assay, using ¹²⁵I- rabbit TgAb as indicator. Tg showed no binding to RF, and a dose-response curve of TgIC in serum could be established. There was a dependence of the Tg-TgAb ratio, TgIC at equilibrium and in antibody excess being detected most efficiently.

The method may serve as an aid in the evaluation of the fate of Tg, TgAb and TgIC following thyroid damage (surgery, radioiodine) and may be extended as a model system in the investigation of immune complexes in connection with autoimmune disorders.

Key words: immune complexes; rheumatoid factor; solid phase immunoradiometric assay; thyroglobulin; ultracentrifugation.

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Since the recognition of Hashimoto's thyroiditis and Graves' disease as autoimmune disorders the study of thyroid diseases has drawn much immunological attention (1, 8, 15, 35, 36, 37). An increased frequency of the first complement factor Clq fixing and complement activating circulating immune complexes (IC) has been found (2, 4, 10, 12, 17, 21), and Takeda & Kriss (33) reported the presence of thyroglobulin-antithyroglobulin immune complexes (TgIC) in seven of 29 patients with Graves' disease.

Furthermore, glomerulonephritis, likely caused by TgIC, has been observed following

radioiodine therapy in Graves' disease (29) and has been experimentally produced in rabbits (38). Finally, TgIC have been supposed to be the causative agent in endocrine exophthalmos (25).

Since thyroglobulin (Tg) is a well-characterized protein molecule, and precise and specific methods for quantitation of Tg (13, 34) and its antibody (TgAb) (6, 31) exist, it would seem to serve as a suitable example for the study of IC containing an autoantigen (Tg). The present study was undertaken to characterize TgIC preformed *in vitro* from purified Tg and TgAb ob-

tained from immune serum from patients with autoimmune thyroid diseases. A preliminary report has been published (22).

MATERIAL AND METHODS

Material

Sera were obtained from four patients with Hashimoto's thyroiditis, one with primary myxoedema, and one with Graves' disease, during treatment. Some of the experiments were performed with serum from only one of the patients with Hashimoto's thyroiditis (Patient 1). Normal serum (NS) with unmeasurable concentrations of Tg and TgAb was obtained from the Blood Bank, Odense Hospital.

Methods

Measurement of Tg and TgAb concentrations in serum was performed as previously described by radioimmunological methods (6, 13).

Preformation of TgIC. Immune sera containing high concentrations of TgAb were used without purification, and different concentrations were obtained by dilution of these TgAb containing patient sera in NS. Human Tg, purified as previously described (9) was added at a fixed amount to the dilutions to yield different antibody – antigen ratios. The ratio of TgAb to Tg, based on antigen binding determinations (6), was 0.1, 0.5, 1.0, 2.0. In experiments with ultracentrifugation, precipitation with polyethylene glycol (PEG), concanavalin A (con A) or con A-Sepharose, TgIC (2.5 μ g Tg/ml) were preformed after addition of a trace amount of ^{125} I-Tg (9) at a specific activity of 30 μ Ci/ μ g. The complexes were shaken for 4 h at 20°C and further incubated for 18 h at 4°C before use.

Ultracentrifugation in sucrose gradient was performed largely as previously described (9), in 10–30% (w/v) sucrose dissolved in 0.5 M phosphate-sodium-chloride buffer (pH = 7.0). After application of 100 μ l of Tg or TgIC, centrifugation was carried out in an International Equipment Company B60 centrifuge for 18 h at

75,000 $\times$ g (22,000 r.p.m.). Fractions of 0.5 ml were collected from the bottom of the polyallomer tubes (14.5 mm $\times$ 9.6 mm), and counted in a well-type gamma-counter (Selectronic®, Denmark). The fractions were weighed and the corresponding S values were processed as described by Steensgaard et al. (32). Furthermore, the serum protein alpha₂-macroglobulin was measured in the fractions by electroimmunoassay (26), and used as a marker of the 19S region.

Precipitation with con A and con A-Sepharose. 100 μ l of each fraction from the sucrose gradient ultracentrifugation was mixed with con A (Pharmacia Fine Chemicals, Sweden) (final concentration 12.5 g/l) and another 100 μ l was mixed with con A-Sepharose (Pharmacia Fine Chemicals) (final concentration 25% suspension). Tubes (Polystyrene 7 $\times$ 11 mm, Nunc, Denmark) were incubated at 20°C for 1 h and centrifuged (1500 $\times$ g, 10 min), whereafter the radioactivity was counted in both supernatant and pellet.

Con A-Sepharose chromatography. Tg or TgIC preformed as described above, trace amount of ^{125} I-Tg added, was applied to con A-Sepharose columns (5-ml syringe (Pharma-Plast, Denmark) containing 2 ml gel). Stepwise elution from 0–20% methyl-alpha-D-glucopyranoside and buffer (Calbiochem, California) was performed and radioactivity counted in the eluates.

Precipitation with PEG was performed using 100 μ l sample (Tg or TgIC) and 400 μ l borate buffer (pH = 8.6) containing 0–20% PEG 6000 (Sigma, USA) in end concentration. The tubes were kept on ice for 18 h, centrifuged for 20 min at 1500 $\times$ g, whereafter the precipitate was washed in the appropriate PEG-buffer. The radioactivity in the final precipitate was counted in a gamma-counter.

Purification of rabbit-TgAb. For use in the rheumatoid factor TgIC assay, thyroglobulin antiserum was raised in rabbits according to Harboe & Ingild (16). The antiserum was applied at 4°C to an affinity column (K 16/40®, Pharmacia Fine Chemicals, Sweden) containing Sephadex G-50 as gel in the lower two-thirds and CNBr-

Sephadex covalently coupled with Tg in the upper one-third (9). The absorbed TgAb was eluted with HCl-glycine buffer, pH = 2.8 neutralization being performed immediately by addition of 5 ml bicarbonate buffer 1 M, pH = 10.

The concentration of rabbit TgAb in the final solution, after concentration on Minicon® (Amicon Corp., MA, USA) was measured by electroimmunoassay (26), using rabbit IgG (Dako Immunoglobulins, Denmark) as standard and 20 μ l anti-rabbit IgG from goat per 10 ml agarose gel (Indubiose, France).

^{125} I-labelling was performed by the chloramine-T method using 50 μ g rabbit TgAb (pH adjusted to 7.0) for 1 mCi ^{125} I (Amersham, Great Britain). Separation of free and protein bound radioactivity was done by a Sephadex G-25 column (K 26/50®, Pharmacia Fine Chemicals, Sweden).

Purification of polyclonal rheumatoid factor IgM (pRF-IgM) and NHS-IgM. Affinity chromatography for the purification of IgM was performed with serum from a patient with rheumatoid arthritis and a pool of normal human serum (NHS) respectively. 6 g CNBr-Sepharose 4B (Pharmacia, Fine Chemicals, Sweden) was prepared with 200 mg rabbit-anti-IgM (Dako Immunoglobulins, Denmark) and used as the top gel of a K 26/50-column (Pharmacia, Sweden) in which the lower two-thirds consisted of Sephadex G-50. 5 ml serum was applied to the column at a flow of 5 ml/h, and before and after this the column was washed with 15 ml of 0.1 M phosphate buffer, pH 7.4 and with 15 ml of 1 M NaCl. Desorption was obtained with HCl-glycine buffer, pH 2.8, and the fractions were neutralized by adding 1 M NaHCO₃. The IgM concentration was measured by electroimmunoassay (26), and the IgM-containing fractions were pooled.

Rheumatoid factor solid phase radiometric assay (pRF-IRMA) (Fig. 1). Two μ g pRF-IgM or normal human IgM was added in 1 ml phosphate buffered saline (pH = 7) to polystyrene tubes (Nunc, Denmark). The tubes were shaken for 18 h at 20°C and decanted. TgIC or relevant controls (200 μ l) and 800 μ l phosphate buf-

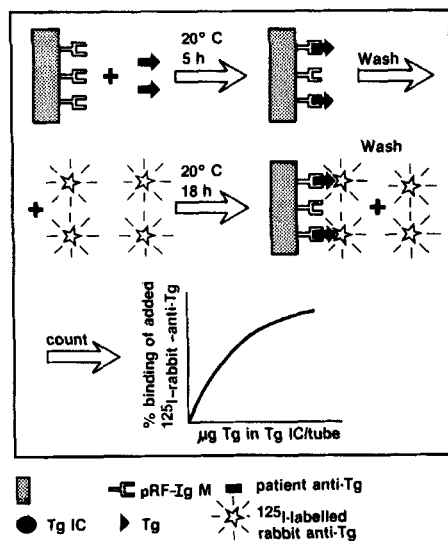


Fig. 1. Illustration of the principle for measurement of TgIC by detection of the TgAb by solid-phase-RF and of Tg by ^{125} I-rabbit anti-Tg.

fered 0.9% NaCl containing bovine serum albumin (1 g/l) and Tween (17.5 ml/l) were added to each tube. The tubes were incubated for 5 h at 20°C, decanted and washed thrice in the same buffer. Finally, 100 ng ^{125} I rabbit TgAb in the same buffer was added and the tubes further incubated for 18 h, washed thrice, and the radioactivity measured.

Affinity studies with human complement factor Clq. Clq was purified by euglobulin precipitation as described by Yonemasu & Stroud (39) and 2 μ g in 1 ml PBS was added and shaken for 4 h at 20°C. Further procedures as for the pRF-IRMA.

RESULTS

The ultracentrifugation profile of Tg as well as TgIC preformed at different ratios between Tg and TgAb from one of the patients with Hashimoto's thyroiditis is shown in Fig. 2. Increasing amounts of TgAb in TgIC caused increasing sedimentation coefficient and increasing heterogeneity of the activity. The size of these complexes was mainly around 25S with both larger and smaller elements, too.

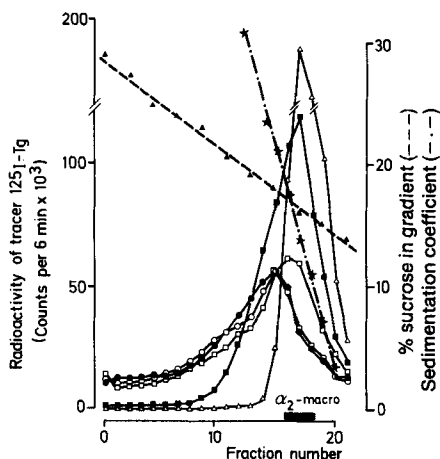


Fig. 2. Ultracentrifugation in sucrose gradient (10–30% w/v) of Tg and TgIC preformed at four different TgAb-Tg ratios, keeping amount of Tg and ^{125}I -Tg constant. The sedimentation coefficients ($\star$) were determined by Steensgaard et al. (32). Only for TgIC at TgAb-Tg 1.0 ($\square$) and 2.0 ($\bullet$) was a clear shift in position of the radioactivity noted. Alpha₂-macroglobulin was used as a marker for 19S (measured by electroimmunoassay). ($\square$) TgAb-Tg = 0.5; ($\blacksquare$) TgAb-Tg = 0.1; ($\triangle$) Tg only. $\blacktriangle$ indicates % sucrose in gradient.

Subjecting the fractions to precipitation studies with con A and absorption with con A-Sepharose not all activity was recovered in the pellet. The mean ratio between activity in supernatant and in pellet from con A precipitation was for 19S Tg 1.56 (range: 1.42–1.66, $n = 4$) and for 25S TgIC 1.96 (range: 1.57–2.3, $n = 3$). The corresponding figures for con A-Sepharose were 0.96 (range 0.66–1.32, $n = 4$) and 1.19 (range: 1.0–1.3, $n = 3$).

The preformed complexes were also subjected to con A-Sepharose column chromatography, which retained completely both Tg and TgIC, expressed as ^{125}I -Tg radioactivity at all antigen antibody ratios. They were neither displaced by methyl- α -D-glucopyranoside, nor by low pH, but only by a strongly alkaline solution (NaOH 0.01 M, pH = 12).

PEG-precipitation studies yielded for each TgIC a precipitation curve from 0–20% PEG. From these the PEG-concentration at which 50% of the radioactivity was precipitated was estimated (Fig. 3A). Tg required a mean of 13.8% (± 0.7 s.d., $n = 6$) of PEG, and TgIC from 5% to 8%, the TgIC in TgAb-excess being pre-

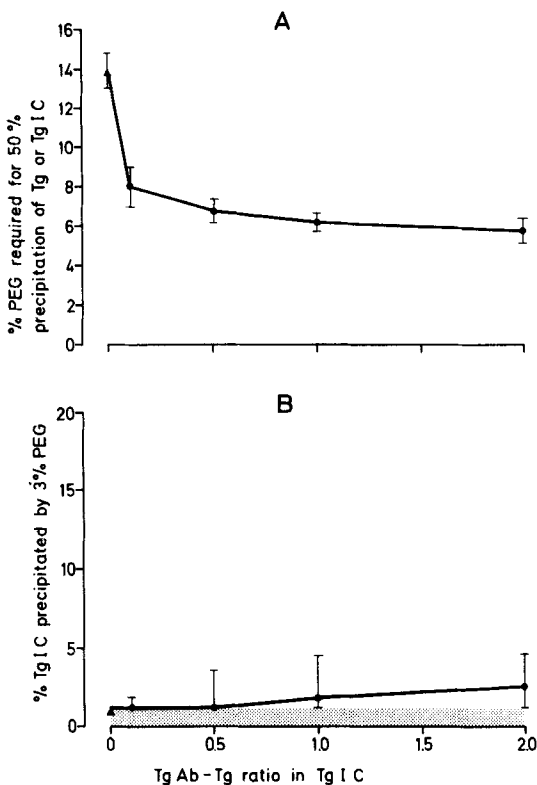


Fig. 3A. Polyethylene glycol (PEG) precipitation of Tg ($\blacktriangle$) and of TgIC ($\bullet$) at four different ratios (0.1–2.0), keeping amounts of Tg and ^{125}I -Tg constant. The mean of results from six patients is shown. Bars indicate $\pm$ s.d.

Fig. 3B. Per cent radioactivity in Tg or TgIC of different ratios precipitated by 3% PEG. Hatched area indicates background level. The mean of results from six patients is shown. Bars indicate range.

cipitable at the lowest PEG-concentration. At a PEG-concentration of 3% the precipitation of TgIC was maximally 5% with a mean of from 1.5–3.5% depending on Tg/TgAb ratio (Fig. 3B).

Fig. 4 shows the binding of TgIC to pRF-IgM as well as the lack of binding to human Clq. At a concentration of $2.5 \mu\text{g}$ Tg/ml serum in the pRF-IRMA a rather constant percentage binding of added radioactivity (40–45%) was obtained from slight antigen excess, through equilibrium to antibody excess, whereas TgIC at large Tg

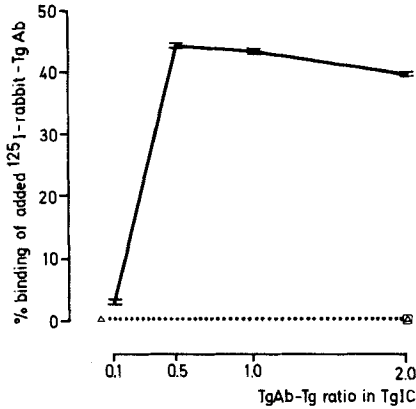


Fig. 4. Binding of human TgIC, using serum from patient 1 for preparation of TgIC at four different TgAb-Tg ratios (0.1-2.0), to solid-phase pRF-IgM. ^{125}I -rabbit-TgAb was used as indicator. Background values (binding to solid-phase normal IgM: 300 ct/min, corresponding to 0.21% bound) were subtracted. Δ Tg; $\square$ TgAb; I range of duplicates.

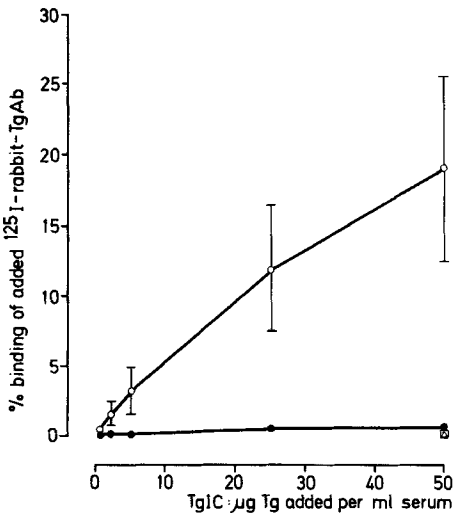


Fig. 5. Dose-response curve of TgIC prepared at a fixed TgAb-Tg ratio of 0.5 using antiserum from patient 1 as source of TgAb. The concentration of TgIC on the abscissa is expressed as μg Tg/ml serum. Open circles and bars denote mean $\pm$ s.d. (= 2 s.e.m.) of four between-day runs in an assay with solid-phase pRF-IgM (see Fig. 1). Background values (binding to solid-phase normal IgM: approximately 300 ct/min, corresponding to 0.18% bound) subtracted. Filled circles denote a similar registration with solid-phase human Clq, showing only negligible binding, Δ Tg: $0.48\% \pm 0.20\%$; $\square$ TgAb $0.29\% \pm 0.06\%$, both at maximal concentrations used.

excess showed only a low binding (approx. 3%). Pure Tg showed no binding at all.

A dose-response curve using TgIC at a TgAb-Tg ratio of 0.5 was constructed from 0-50 μg Tg/ml serum (Fig. 5). Increasing binding with increasing TgIC concentration was seen. The detection limit based on the imprecision in O-samples was approximately 4 mg Tg in TgIC/ml serum.

DISCUSSION

Characterization of the precipitation by PEG of Tg and TgIC, preformed with rabbit Tg-antibody, has previously been done by Creighton et al. (5). Even though affinity constants of the antigen-antibody reactions of the heterologous rabbit and homologous human antibody were wide apart, and although protein concentrations were different, results were comparable. This was compatible with results of Helsing (19) showing that specific antibodies of different affinities for serum albumin precipitated similarly in a polysaccharide media. He concluded that the precipitation was due to steric exclusion of immune reactants (20). PEG may serve as an aid in the separation of Tg from TgIC but is not sufficient as the only method due to overlaps, the precipitation of Tg beginning at a concentration where TgIC is not fully precipitated. The use of 2-3% PEG as a pre-precipitation procedure in connection with methods for quantitation of IC, would only measure a limited amount of the TgIC present (max. 5%).

The same argument concerning separation of Tg and TgIC is true for ultracentrifugation in sucrose gradient, where TgIC showed a gradual increase in molecular weight. Furthermore, this is a very time-consuming method. Takeda & Kriss (33), for the initial step in their TgIC assay, used gel filtration, which is comparable as a separation procedure to ultracentrifugation. The ultracentrifugation method may, however, prove of importance in characterizing the composition of the complexes (23).

Some IC have previously been shown to be precipitable by con A, depending on the antigen content of glycoprotein (18). As Tg is a glycoprotein and free con A requires two or more

binding sites for precipitation (3), it was assumed that TgIC formation lead to differences in the binding to con A, by blocking one or more sites. Although we found a more pronounced precipitation of free Tg the difference was insignificant. IgG itself also has a weak affinity for con A, but the very firm binding of Tg and TgIC to the con A-Sepharose columns was not readily explainable, but might suggest a very high association constant of Tg.

The complete lack of binding between complement factor Clq and TgIC of human origin has previously been observed by others (7, 27, 30). It has also been suggested from *in vivo* studies that TgIC might have affinity for pRF (7). This was confirmed in the present study. pRF has previously been used as ligand in antigen unspecific methods for IC measurement by detection of the IgG of the complex (14). In the present method the detection by radioactivity was applied to the antigenic part of the immune complex, probably more suitable sterically than the antibody part which was attached to pRF already (Fig. 1), an approach comparable to the one used for Clq (28). Furthermore, rabbit anti-Tg was used as this is known to have binding sites towards a considerably larger amount of antigenic determinants of Tg than human anti-Tg (30, 31).

The pRF-IRMA could detect TgIC quantitatively at equilibrium or in antibody excess, due to rather constant binding irrespective of the antigen-antibody ratio. Only a change towards high antigen excess might give falsely low values. Whether this occurred could be elucidated by studying sequential specimens during thyroid surgery (11), where an antigen excess, due to release of antigen from the damaged thyroid gland is provoked. Disappearance of serum TgAb gives indirect evidence of immune complex formation. A similar situation is described in a case of glomerulonephritis complicating radioiodine therapy (29). This approach might consequently serve as an example for the study of IC containing other autoantigens, and could be applied to the antigen for thyroid-stimulating immunoglobulins (24), or other thyroidal antigens. This would be particularly interesting as both specific TgIC, and complement fixing

IC unrelated to Tg have recently been demonstrated in increased frequency in patients with thyroid diseases (27).

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REFERENCES

- Allison, A. C.: Self-tolerance and autoimmunity in the thyroid. *New Engl. J. Med.* 295, 821-827, 1976.
- Brohee, D., Delespesse, G. & Bonnyns, M.: Circulating immune complexes in thyroid disease. *Ann. Endocrinol.* 39, 20A, 1978.
- Bøg-Hansen, T., Prahl, P. & Løwenstein, H.: A set of analytical electrophoresis experiments to predict the results of affinity chromatographic separations: Fractionation of allergens from cow's hair and dander. *J. Immunol. Method* 22, 293-307, 1978.
- Calder, E. A., Penhale, W. J., Barnes, E. W. & Irvine, W. J.: Evidence for circulating immune complexes in thyroid disease. *Br. Med. J.* 2, 30-31, 1974.
- Creighton, W. D., Lambert, P. H. & Miescher, P. A.: Detection of antibodies and soluble antigen-antibody complexes by precipitation with polyethylene glycol. *J. Immunol.* 111, 1219-1227, 1973.
- Date, J., Feldt-Rasmussen, U., Hyltoft Petersen, P. & Beck, K.: An improved coprecipitation assay for determination of thyroglobulin antibodies. *Scand. J. Clin. Lab. Invest.* 40, 37-44, 1980.
- Davis, J. S. & Torrigiani, G.: Effect of rheumatoid factor on in vivo distribution of aggregated human IgG and antigen-antibody complexes. *Proc. Soc. Exp. Biol. (N.Y.)* 125, 772-776, 1967.
- Doniach, D. & Florin-Christensen, A.: Autoimmunity in the pathogenesis of endocrine exophthalmos. *Clin. Endocrinol. Metab.* 1, 34-35, 1975.
- Feldt-Rasmussen, U.: Purification of human thyroglobulin for radioimmunoassay and testing by ultracentrifugal analysis and immunoelectrophoresis. *J. Immunol. Method.* 21, 295-303, 1978.
- Feldt-Rasmussen, U., Bech, K., Nielsen, H. & Date, J.: Circulating thyroglobulin, antithyroglobulin and immune complexes in patients with thyroid disease. *Ann. Endocrinol.* 38, 88A, 1977.

11. Feldt-Rasmussen, U., Petersen, P. H., Nielsen, H., Date, J. & Madsen, C. M.: Thyroglobulin of varying molecular sizes with different disappearance rates in plasma following subtotal thyroidectomy. *Clin. Endocrinol.* 9, 205-214, 1978.
12. Feldt-Rasmussen, U., Bech, K., Date J., Thyme, S., Hannover Larsen, J. & Nielsen, H.: Anticomplementary activity in diffuse and nodular goiters. *Acta Pathol. Microbiol. Scand. Sect. C* 87, 365-370, 1979a.
13. Feldt-Rasmussen, U., Hyltoft Petersen, P. & Date, J.: Sex and age correlated reference values of serum thyroglobulin measured by a modified radioimmunoassay. *Acta Endocrinol.* 90, 440-450, 1979b.
14. Glickmann, G., Svehag, S.-E. & Nielsen, H.: A solid-phase, polyclonal IgM-RF binding assay for circulating immune complexes. *Acta Path. Microbiol. Scand., Sect. C* 87, 121-129, 1979.
15. Hall, R.: Immunological aspects of thyroid function. *New Engl. J. Med.* 266, 1204-1211, 1962.
16. Harbo, N. & Ingild, A.: Immunization, isolation of immunoglobulins, estimation of antibody titre. *Scand. J. Immunol.* 2, Suppl. 1, 161-163, 1973.
17. van der Heide, D., Daha, M. R., Bolk, J., Bussemaker, J. K. & van Es, L. A.: TSI and circulating immune complexes in patients with Graves' disease. *Ann. Endocrinol.* 40, 4A, 1979.
18. Heimer, R. & Klein, G.: The affinity of soluble immune complexes for concanavalin A. *Scand. J. Immunol.* 7, 315-320, 1978.
19. Hellsing, K.: Immune reactions in polysaccharide media, experiments with specific antibodies of different affinities for serum albumin. *Biochem. J.* 114, 151-155, 1969.
20. Hellsing, K.: Immune reactions in polysaccharide media, the composition of the antigen-antibody complexes in the precipitin reaction. *Biochem. J.* 114, 141-144, 1969.
21. Hopf, V., Hutteroth, T. H. & Meyer zum Büschenfelde, K.-H.: Detection of circulating immune complexes in patients with Graves' disease. *Ann. Endocrinol.* 39, 20A, 1978.
22. Husby, S., Feldt-Rasmussen, U., Blåbjerg, O., Date, J., Nielsen, H. & Svehag, S.-E.: *In vitro* characterization of thyroglobulin immune complexes. *Annal. Endocrinol.* 40, 6A, 1979.
23. Husby, S., Steensgaard, J., Feldt-Rasmussen, U. & Date, J.: Ultracentrifugation pattern of thyroglobulin immune complexes. (In preparation, 1980).
24. Knox, A. J. S., Westarp, C. von, Row, V. V. & Volpé, R.: Evidence that the antigens to the human thyroid stimulators of Graves' disease are located in the thyroid gland. In: Robbins, J. & Braverman, L. E. (eds.): *Thyroid research*, pp. 417-420. Excerpta Medica, Amsterdam-Oxford, 1976.
25. Kriss, J. P., Konishi, J. & Herman, M.: Studies on the pathogenesis of Graves' ophthalmopathy (with some related observations regarding therapy). *Recent Progr. Horm. Res.* 31, 533-566, 1975.
26. Laurell, C. B.: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Analy. Biochem.* 15, 45-52, 1966.
27. Mariotti, S., DeGroot, L. J., Scarborough, D. & Medof, E.: Study of circulating immune complexes in thyroid diseases: Comparison of Raji cell radioimmunoassay and specific thyroglobulin-antithyroglobulin radioassay. *J. Clin. Endocrinol. Metab.* 49, 679-686, 1979.
28. Nielsen, H., Glickmann, G. & Svehag, S.-E.: Circulating immune complexes in patients with varicella-zoster infection. Identification of complex bound antigen. In: Peeters, H. (ed): *Protides of the biological fluids*, Pergamon Press, 26, 187-189, 1978.
29. Ploth, D. W., Fitz, A., Schnetzler, D., Seidenfeld, J. & Wilson, S. B.: Thyroglobulin anti-thyroglobulin immune complex glomerulonephritis complicating radioiodine therapy. *Clin. Immunol. Immunopathol.* 9, 327-334, 1978.
30. Roitt, I. M., Torrigiani, G. & Doniach, D.: Immunochemical studies on the thyroglobulin autoantibody system in human thyroiditis. *Immunology* 15, 681-696, 1968.
31. Salabé, G. B., Salabé, H., Dominici, R., Davoli, C. & Andreoli, M.: Radioimmunoassay for human antithyroglobulin antibodies. II. Determination of antigen binding capacity. *J. Clin. Endocrinol. Metab.* 39, 1125-1132, 1974.
32. Steensgaard, J., Møller, N. P. H. & Funding, L.: Rate zonal centrifugation: Quantitative aspects. In: Birnie, C. D. & Rickwood, D. (eds.): *Centrifugal separations in molecular and cell biology*, pp. 115-167. Butterworths, London, 1978.
33. Takeda, Y. & Kriss, J. P.: Radiometric measurement of thyroglobulin antithyroglobulin immune complexes in human serum. *J. Clin. Endocrinol. Metab.* 44, 46-55, 1977.
34. Van Herle, A. J., Uller, R. P., Matthews, N. L. & Brown, J.: Radioimmunoassay for measurement of thyroglobulin in human serum. *J. Clin. Invest.* 52, 1320-1327, 1973.
35. Volpé, R., Farid, N. R., von Westarp, C. & Row, V. V.: The pathogenesis of Graves' disease and Hashimoto's thyroiditis. *Clin. Endocrinol.* 3, 239-261, 1974.
36. Volpé, R.: The pathogenesis of Graves' disease: An overview. *Clin. Endocrinol. Metab.* 7, 3-29, 1978.
37. Volpé, R., Okita, N. & Kidd, A.: The role of the lymphocytes in the aetiology of Graves' disease. In Pinchera, A. (ed): *The Sero Symposium on autoimmunity in the endocrine system*, Academic Press, 1980 (In press).
38. Weigle, W. O. & High, G. J.: The behaviour of autologous thyroglobulin in the circulation of rabbits immunized with either heterologous or altered homologous thyroglobulin. *J. Immunol.* 98, 1105-1114, 1967.
39. Yonemasu, K. & Stroud, R. M.: Clq: rapid purification method for preparation of monospecific antisera and for biochemical studies. *J. Immunol.* 106, 304-313, 1971.

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