Clin. exp. Immunol. (1979) 36, 379–383.

Circulating immune complexes in various thyroid diseases

D. BROHEE, G. DELESPESSE, M. J. DEBISSCHOP & M. BONNYNS **Departments of Medicine and Immunology, Saint-Pierre Hospital and Laboratory of Experimental Medicine, Brussels Free University, Brussels, Belgium*

(*Accepted for publication* 14 *December* 1978)

SUMMARY

In a study of 171 patients with various thyroid diseases, circulating immune complexes (CIC), measured by a C1q solid phase radioassay, were detected in 26% of the patients as compared to 8% of the control subjects. CIC were found in 33–55% of the patients with a well defined thyroid autoimmune disorder (Hashimoto's goitre, asymptomatic thyroiditis, spontaneous myxoedema and Graves' disease) and also in the same proportion of patients with diffuse goitre. CIC were correlated to the presence of serum antibodies to microsomal thyroid antigen but not to their titre. No relationship was observed between CIC and the age or sex of the patients and the presence of exophthalmos, or between CIC and the different thyroid function tests or serum anti-thyroglobulin antibodies. CIC were found in untreated patients as well as in those treated with prednisone, methimazole or thyroxine.

INTRODUCTION

The incidence of circulating immune complexes has been extensively studied in various clinical conditions including autoimmune diseases (Wells, 1976). The role of immune complexes in autoimmune thyroid disorders was suggested by immunofluorescent studies showing granular deposits of immunoglobulins and complement in the connective tissue stroma and in follicular basement membrane of thyroid glands from patients with Graves' disease (Werner *et al.*, 1972; Kalderon, Bogaars & Diamond, 1973) and Hashimoto's thyroiditis (Kalderon *et al.*, 1973). Electron microscopy confirmed these findings (Kalderon *et al.*, 1973; Kalderon & Bogaars, 1977). Circulating immune complexes (CIC) measured by anti-complementary assays were first reported by Calder *et al.* (1974) in the sera of patients with Hashimoto's goitre, primary hypothyroidism and Graves' disease. Recent studies using different methods have confirmed the presence of CIC in autoimmune thyroid diseases (Barkas *et al.*, 1976; Cano *et al.*, 1976; Takeda & Kriss, 1977).

A pathogenic role for CIC in thyroid autoimmune diseases was suggested by the observation that sera of patients with Hashimoto thyroiditis contained 19S IgG complexes able to recruit and arm normal lymphocytes against thyroglobulin-coated chicken red blood cells (Calder *et al.*, 1973b; Calder, McLeman & Irvine, 1973a). The importance of CIC occurring in autoimmune thyroiditis was further emphasized by reports of glomerulonephritis with kidney deposits of complement, immunoglobulins and thyroid antigens (O'Reagan *et al.*, 1976; Ploth *et al.*, 1978). CIC were also suspected of playing a role in the pathogenesis of exophthalmos, as a result of the demonstration that thyroglobulin-antithyroglobulin immune complexes were bound to extraocular muscle membranes (Konishi, Herman & Kriss, 1974).

The aim of the present study was the direct measurement of CIC, not only in autoimmune thyroiditis but also in various thyroid diseases by using a C1q solid phase radioassay. We present evidence that this assay is useful for the diagnosis of a thyroid autoimmune process.

Correspondence: Dr G. Delespesse, Department of Immunology, Saint-Pierre University Hospital, 1000 Brussels, Belgium.

0099-9104/79/0060-0379 $02.00

MATERIALS AND METHODS

Patients. 171 patients (150 females and twenty-one males, aged from 17 to 80 years, mean 42 years) were investigated in the Departments of Medicine and Endocrinology, Sainte-Pierre Hospital. Thyroid diagnosis was by classical clinical examination and thyroid tests. The study was double-blind: CIC determination was carried out independently by G. Delespesse and M.J. Debisschop, and thyroid diagnosis was assessed by M. Bonnyns and D. Brohee. No patient suffered from a known intercurrent infectious disease or from any other autoimmune disorder. The patients were compared to 100 control blood donors.

Detection of CIC. Immune complexes were measured by a modification of the method of Hay, Nineham & Roitt (1976). Briefly, purified human C1q (10 μg/ml in phosphate buffered saline, PBS) was used to coat polystyrene tubes. 50 μl of test sera were incubated with 150 μl EDTA 0·2 M pH 7·2 for 30 min at 37°C and then cooled in an ice bath. Fifty μl of EDTA-treated sera were added to the C1q-coated tubes together with 150 μl PBS containing 0·02% Tween 20. Each test was performed in duplicate. Coated tubes containing 0·2 ml PBS Tween were used as background controls. The tubes were incubated for 1 hr at 37°C and overnight at 4°C. Unbound proteins were then removed by washing with PBS. Immune complexes bound to the C1q-coated tubes were detected by incubating the tubes with 0·2 ml ^{125}I-protein A from *Staphylococcus aureus*. The latter was purchased from Pharmacia (Sweden) and radiolabelled according to the method of Klinman & Taylor (1969). The radioactivity added to each tube was 100,000 ct/min. After incubation overnight at room temperature, the tubes were washed with PBS and counted in a gamma counter. Bound radioactivity was a measure of the immune complexes in the tested serum. In each assay, sera from six to ten healthy blood donors were used as normal controls. The mean value+2 s.d. of the radioactivity bound by these control sera was used as the limit of positivity.

Thyroid tests. The thyroid function was estimated by ^{131}I uptake and ^{99m}Tc or ^{131}I scintiscans, T_3 resin uptake (Thiopac 3), thyroxine (T_4) radioimmunoassay (RIA) (Cheung & Slaunwight, 1976), triiodothyronine (T_3) RIA (Hüfner & Hesch, 1973) and thyrotropin (TSH) RIA (Wide & Porath, 1966). Serum TSH was also measured before, and 15 and 30 min after i.v. injection of 200 μg of thyrotropin-releasing hormone (TRH). Anti-thyroglobulin antibodies (TGA) were determined by RIA (Delespesse *et al.*, 1976) and antibodies to thyroid microsomal antigen (CFA) by immunofluorescence.

RESULTS

Thyroid diseases were subdivided into autoimmune (n = 100) and non-immune (n = 71) cases. The first group comprised patients with Graves' disease (n = 25), Hashimoto's thyroiditis (n = 4), asymptomatic thyroiditis (n = 37), spontaneous myxoedema (n = 5) and goitre with low levels of thyroid antibodies (n = 29). In the non-immune group were included patients with diffuse goitre (n = 23), multinodular goitre (n = 26), cold nodule (n = 16) and hot nodule (n = 6). Asymptomatic thyroiditis is characterized by a lymphocytic thyroiditis developing without clinical signs in a subject without a past history of thyroid disease and with a thyroid gland of normal size. The TRH test in asymptomatic thyroiditis was normal or showed an increased response, with either a normal or elevated basal serum TSH level. Patients having a goitre with low levels of thyroid antibodies had normal T_4 and T_3 levels; the TRH test was normal in most of the patients but in some cases, the TSH response was increased.

Thyroid antibodies (TGA or CFA) were detected in 114 patients (67%), TGA in 112 (65%) and CFA in 41 (24%). Besides thyroid antibodies associated with autoimmune thyroid disease, TGA or CFA were also found in cold (eleven out of sixteen) and hot nodules (three out of six).

The frequency of CIC in control subjects and in patients is shown in Table 1. CIC were statistically related to thyroid disorders and to thyroid autoimmunity. The incidence of CIC in the different groups of thyroid diseases is given in Table 2. CIC were observed in one in three to one in two of the four main varieties of autoimmune thyroid disorders, but also, surprisingly, in the same proportion of cases of

TABLE 1. Frequencies of CIC in patients and in control subjects

	n	Positive CIC	Percentage	P
Control subjects	100	8	8	$< 0·001$
Thyroid patients	171	44	26	$< 0·001$
With thyroid autoimmunity	100	33	33	$< 0·01$
Without thyroid autoimmunity	71	11	16	$> 0·1$

TABLE 2. Incidence of CIC in different thyroid diseases

	N	Positive CIC	Percentage	*P**
Graves' disease	25	7	28	< 0·02
Hashimoto's goitre	4	2		n.s.
Spontaneous myxoedema	5	4		= 0·001
Asymptomatic thyroiditis	37	15	40	< 0·001
Goitre with thyroiditis	29	5	17	n.s.
Diffuse goitre	23	7	30	< 0·01
Nodular goitre	26	1	4	n.s.
Cold nodule	16	1	6	n.s.
Hot nodule	6	2		n.s.

* Compared to the CIC frequency in control group (8%).

diffuse goitre. Patients with diffuse goitre and positive CIC were younger than the others and the duration of their goitre was shorter than in the group without CIC; they did not have a family history of goitre or autoimmunity, and there was no clinical difference between the positive and the negative CIC groups. The frequency of CIC in the others groups was lower than 20% if data on the hot nodule is excluded as confirmation from more cases is needed. It should be noted that CIC were not significantly related to goitre with superimposed thyroiditis.

CIC were statistically associated with the presence of CFA but not TGA (Table 3). Further analysis failed to show any relationship between the level of CIC and the titre of CFA. There was no correlation between CIC and sex, age and the presence of exophthalmos or between CIC and serum cholesterol, T_4, T_3 and TSH levels. Finally, CIC were found in the sera of untreated patients as well as in those treated with prednisone, methimazole or thyroxine.

DISCUSSION

It is well known that studies dealing with the incidence of CIC in a given disease are greatly influenced by the type of immune complex assay and by patient selection. In the present work, the solid-phase C1q assay was used to test sera from 100 control subjects and 171 patients with well-documented thyroid disease. The endpoint of the method is IgG1 specific; indeed, only immune complexes containing sufficient amounts of either IgG1, IgG3 or IgM bind C1q, whereas protein A is specific for the IgG subclasses 1, 2 and 4 (Cohen, 1975). The IgG1 specificity of the assay explains the absence of false positive results in sera containing heparin, DNA or endotoxin (Tung *et al.*, 1978). These substances bind C1q and can alter the C1q deviation test (Sobel, Bokisch & Müller-Eberhard, 1975) and the radiolabelled C1q binding assay (Woodroffe *et al.*, 1977). False positive results due to aggregated immunoglobulins would seem to be unlikely since there was no heat decomplementation and handling of the sera was the same for control and thyroid patients. However, with our method, false negative results cannot be

TABLE 3. Relationship between thyroid antibodies and CIC in 171 patients

Thyroid antibodies	*N*	Positive CIC	Percentage	*P*
TGA or CFA	119	34	28	n.s.
TGA	112	30	27	n.s.
CFA	41	16	39	< 0·05

excluded as immune complexes may be formed by immunoglobulins A, E and M (Werner *et al.*, 1972; Kalderon & Bogaars, 1977).

Positive CIC were found in 8% of control sera, in 16% of sera from patients with non-immune thyroid diseases ($P>0{\cdot}1$) and in 33% of sera from autoimmune thyroid disorders ($P<0{\cdot}001$). These findings, in agreement with those reported previously (Calder *et al.*, 1974; Cano *et al.*, 1976; Takeda & Kriss, 1977), suggest that CIC constitute a good marker of an autoimmune thyroid process. There are, however, two major exceptions to this view: a normal incidence of CIC was found in goitre with low levels of thyroid antibodies, while, on the other hand, an abnormally high incidence of CIC was present in diffuse goitre ($P<0{\cdot}01$). The first observation was not surprising as goitre was the main disease and thyroiditis only a superimposed mild phenomenon, in contrast with the situation found in the four main varieties of autoimmune thyroid diseases (Hashimoto's thyroiditis, asymptomatic thyroiditis, spontaneous myxoedema and Graves' disease) where the autoimmune process plays the predominant pathogenic role. In the case of the high incidence of CIC in diffuse goitre without serum anti-thyroid antibodies, this situation might correspond to an early phase of immune complex formation characterized by the absence of free thyroid antibodies trapped for CIC formation. This phenomenon has been observed in experimental thyroiditis (Clagett, Wilson & Weigle, 1974).

Our finding that CIC are significantly associated with CFA and not with TGA is in agreement with the findings of Cano *et al.* (1976). By contrast, Calder *et al.* (1974) and Takeda & Kriss (1977) reported a relation between CIC and TGA. These apparent discrepancies may be explained by the different methods used. Indeed, it is known that CIC detected in thyroid diseases are heterogeneous in size, varying from 19S to 7S (Calder *et al.*, 1973a; 1974; Cano *et al.*, 1976) and that TGA fix complement weakly compared to CFA. Therefore, complement-dependent assays should favour the detection of high molecular weight immune complexes containing CFA (Hughes, 1977). As CIC were not detected in all patients with autoimmune thyroiditis, it was interesting to assess their possible association with another clinical or biological parameter. In this respect, there was no correlation with exophthalmos, with any thyroid function test or with the type of treatment. In regard to the possible influence of age on the CIC frequency in a normal population (Delespesse & Debisschop, in preparation), it is important to note that our study was not biased as the patients with and without CIC had the same age and sex distribution.

In conclusion, the C1q solid phase radioassay for CIC seems to be a good test for the detection of an autoimmune thyroid process in a cross-sectional study. The pathogenic significance of circulating immune complexes is as yet unclear. Longitudinal evaluation of CIC levels in the patients and identification of the antigen present in the immune complexes should lead to a better understanding.

This work was supported by grant 3·9001 from the Fonds de la Recherche Scientifique Médicale of Belgium. The authors wish to thank Mrs R. De Munck and Mrs M. Noël from the Department of Endocrinology for their cooperation in collecting the clinical data.

REFERENCES

Barkas, T., Al-Khateeb, S.F., Irvine, W.J., Davidson, N.McD. & Roscoe, P. (1976) Inhibition of antibody-dependent cell-mediated cytotoxicity (ADCC) as a means of detection of immune complexes in the sera of patients with thyroid disorders and bronchogenic carcinoma. *Clin. exp. Immunol.* **25**, 270.

Calder, E.A., McLeman, D. & Irvine, W.J. (1973a) Lymphocyte cytotoxicity induced by preincubation with serum from patients with Hashimoto thyroiditis. *Clin. exp. Immunol.* **15**, 467.

Calder, E.A., Penhale, W.J., Barnes, E.W. & Irvine, W.J. (1974) Evidence of circulating immune complexes in thyroid disease. *Brit. Med. J.* ii, 30.

Calder, E.A., Penhale, W.J., McLeman, D., Barnes, E.W. & Irvine, W.J. (1973b) Lymphocyte-dependent antibody-mediated cytotoxicity in Hashimoto thyroiditis. *Clin. exp. Immunol.* **14**, 153.

Cano, P.O., Chertman, M.M., Jerry, L.M. & McKenzie, J.M. (1976) Circulating immune complexes in Graves' disease. *Endocr. Res. Comm.* **3**, 307.

Cheung, M.C. & Slaunwight, W.R. (1976) Use of polyethylene glycol in separating bound from unbound ligand in radioimmuno assay of thyroxine. *Clin. Chem.* **22**, 299.

Clagett, J.A., Wilson, C.B. & Weigle, W.O. (1974) Interstitial immune complex thyroiditis in mice. The role of autoantibody to thyroglobulin. *J. exp. Med.* **140**, 1439.

Cohen, S. (1975) Antibody structure. *Disorders of protein metabolism* (ed. by G. K. McGowan and G. Walters). *J. clin. Path.* **28**, (suppl. 6), 1.

Delespesse, G., Hubert, C., Gausset, P. & Govaerts, A. (1976) Radioimmunoassay for human antithyroglobulin antibodies of different immunoglobulin classes. *Horm. metab. Res.* **8**, 50.

Hay, F.C., Nineham, L.J. & Roitt, I.M. (1976) Routine

assay for the detection of immune complexes of known immunoglobulin class using solid phase C1q. *Clin. exp. Immunol.* **24**, 396.

HÜFNER, M. & HESCH, R.D. (1973) A comparison of different compounds for TBG-blocking used in radioimmunoassay for tri-iodothymine. *Clin. Chem. Acta*, **44**, 101.

HUGHES, G.R.V. (1977) Immune complex disease and the complement system. *Connective Tissue Diseases* (ed. by G. R. V. Hughes), p. 226. Blackwell, Oxford.

KALDERON, A.E. & BOGAARS, H.A. (1977) Immune complex deposits in Graves' disease and Hashimoto's thyroiditis. *Amer. J. Med.* **63**, 729.

KALDERON, A.E., BOGAARS, H.A. & DIAMOND, I. (1973) Ultrastructural alterations of the follicular basement membrane in Hashimoto's thyroiditis. Report of eight cases with basement membrane deposits. *Amer. J. Med.* **55**, 485.

KLINMAN, N.R. & TAYLOR, R.B. (1969) General methods for the study of cells and serum during the immune response. The response to dinitrophenyl in mice. *Clin. exp. Immunol.* **4**, 473.

KONISHI, J., HERMAN, M.M. & KRISS, J.P. (1974) Binding of thyroglobulin and thyroglobulin-antithyroglobulin immune complex to extraocular muscle membrane. *Endocrinology*, **95**, 434.

O'REAGAN, S., FONG, J.S.C., KAPLAN, B.S., DE CHADAVERIAN, J.P., LAPOINTE, N. & DRUMMOND, K.N. (1976) Thyroid antigen–antibody nephritis. *Clin. Immunol. Immunopath.* **6**, 341.

PLOTH, D.W., FITZ, A., SCHNETZLER, D., SEIDENFELD, J. & WILSON, C.B. (1978) Thyroglobulin-anti-thyroglobulin immune complex glomerulonephritis complicating radioiodine therapy. *Clin. Immunol. Immunopath.* **9**, 327.

SOBEL, A.T., BOKISCH, V.A. & MÜLLER-EBERHARD, H.J. (1975) C1q deviation test for the detection of immune complexes, aggregates of IgG and bacterial products in human serum. *J. exp. Med.* **142**, 139.

TAKEDA, Y. & KRISS, J.P. (1977) Radiometric measurement of thyroglobulin-antithyroglobulin immune complex in human serum. *J. clin. Endocrinol. Metab.* **44**, 46.

TUNG, K.S.K., WOODROFFE, A.J., AHLIN, T.D., WILLIAMS, R.C., JR. & WILSON, C.B. (1978) Application of the solid phase C1q and Raji cell radioimmune assays for the detection of circulating immune complexes in glomerulonephritis. *J. clin. Invest.* **62**, 61.

WELLS, J.V. (1976) Immune complex disorders. Immune mechanisms in tissue damage. *Basic and Clinical Immunology* (ed. by H.H. Fudenberg, D.P. Stites, J.L. Caldwell and J.V. Wells), p. 232. Lange Medical Publications, Los Altos, USA.

WERNER, S.C., WEGELIUS, O., FIERER, J.A. & HSU, K.C. (1972) Immunoglobulins (E, M, G) and complement in thyroid of Graves' disease. *New Engl. J. Med.* **287**, 421.

WIDE, L. & PORATH, J. (1966) Radioimmunoassay of proteins with the use of sephadex coupled antibodies. *Biochim. biophys. Acta*, **130**, 257.

WOODROFFE, A.J., BORDER, W.A., THEOPHILOPOULOS, A.N., GÖTZE, D., GLASSOCK, R.J., DIXON, F.J. & WILSON, C.B. (1977) Detection of circulating immune complexes in patients with glomerulonephritis. *Kidney Int.* **12**, 268.