Clin. exp. Immunol. (1982) **47**, 336–344.

Studies on thyroid cell surface antigens using cultured human thyroid cells

G. F. FENZI,* L. BARTALENA,* L. CHIOVATO,† C. MARCOCCI,* C. M. ROTELLA,‡ R. ZONEFRATI,‡ R. TOCCAFONDI‡ & A. PINCHERA†

** Cattedra di Patologia Medica 2, University of Pisa, † Cattedra di Medicina Costituzionale ed Endocrinologia, University of Pisa, and ‡ Clinica Medica 3, University of Florence, Italy*

(Accepted for publication 11 September 1981)

SUMMARY

Human thyroid cells in primary culture were used for studies of thyroid cell surface antibodies in patients with thyroid autoimmune disorders. Radioiodinated IgG preparations containing thyroid microsomal antibody (TMAb), thyroid stimulating antibody (TSAb) and/or thyroglobulin antibody (TgAb) were tested for binding to thyroid cells. Binding was observed with radioiodinated IgG from patients with Graves' disease, Hashimoto's thyroiditis and idiopathic myxoedema containing TMAb, irrespective of the presence of TSAb and TgAb, while negative results were obtained with normal IgG. A dose-dependent inhibition of binding to thyroid cells was produced by the addition of the corresponding unlabelled IgG preparations. Evidence for tissue specificity was provided by the absence of binding to human skin fibroblasts used as controls. Preabsorption with human thyroid microsomes completely abolished the binding to thyroid cells of a radioiodinated TMAb positive IgG preparation, while only incomplete removal of the reactivity to thyroid microsomes was produced by preabsorption with thyroid cells. These data suggest that some but not all microsomal antigenic determinants are expressed on the thyroid cell surface. Binding to thyroid cells was also observed with purified TgAb, indicating that thyroglobulin antigenic determinants are present on the surface of thyroid cells. No evidence of binding was obtained with a TSAb positive Graves' IgG preparation with undetectable TMAb and TgAb. Unlabelled IgG preparations containing TMAb from patients with either Hashimoto's thyroiditis or idiopathic myxoedema were shown to inhibit the binding to thyroid cells of radioiodinated TMAb positive Graves' IgG and vice versa. These data indicate that antibodies present in these thyroid autoimmune disorders share common thyroid cell surface antigens. However, the binding of radioiodinated IgG from a patient with idiopathic myxoedema was only partially inhibited by Graves' or Hashimoto's IgG, suggesting that some of the thyroid cell surface antibodies of idiopathic myxoedema may not be detectable in other thyroid autoimmune disorders.

INTRODUCTION

Direct evidence for circulating autoantibodies to thyroid cell surface antigen(s) was previously provided by immunofluorescence on viable human thyroid cells (Fagraeus & Jonsson, 1970) and by mixed haemadsorption using monolayer cultures (Jonsson & Fagraeus, 1969). An indirect im-

Correspondence: Gianfranco Fenzi, M.D., Cattedra di Patologia Medica 2, University of Pisa, via Roma, 67, 56100 Pisa, Italy.

0009-9104/82/0200-0336$02.00

munofluorescence method on monolayer cell cultures has also been used by Khoury *et al.* (1980) for the demonstration of these antibodies. Thyroid cell surface antibodies (TCSAb) have been recently detected by a competitive binding radioassay developed in our laboratory (Toccafondi *et al.*, 1980b). This method is based on the inhibition by unlabelled IgG of binding of radioiodinated IgG from patients with thyroid autoimmune disorders to cultured human thyroid cells. The presence of these antibodies appeared to be highly correlated with that of thyroid microsomal antibodies (TMAb), suggesting that the microsomal antigen might be expressed on the thyroid cell surface (Khoury *et al.*, 1980; Pinchera *et al.*, 1980b).

A thyroid plasma membrane component is believed to give rise to the thyroid-stimulating antibody (TSAb) present in the sera of patients with Graves' disease (Pinchera *et al.*, 1980a; Pinchera *et al.*, 1980c), but the nature of this antigen has not yet been defined. In previous studies (Pinchera *et al.*, 1980b; Toccafondi *et al.*, 1980a) cultured human thyroid cells were shown to have cell surface components reacting with TSAb, since intracellular accumulation of cyclic AMP (cAMP) could be demonstrated after addition of TSAb IgG preparations.

The aim of the present study was to further elucidate the nature of the antigen(s) present on thyroid cell surface. For this we investigated the binding to human thyroid cells of radiolabelled IgG prepared from sera of patients with thyroid autoimmune disorders containing TSAb, TMAb and/or thyroglobulin antibody (TgAb). The interference produced on this binding by unlabelled IgG preparations containing antibodies directed against different thyroid antigens was also studied.

MATERIALS AND METHODS

Preparation of IgG. IgG was prepared from sera of patients with thyroid autoimmune disorders and from normal subjects by the method of Baumstark, Laffin & Bordawill, (1964) with minor modifications (Fenzi *et al.*, 1978), using the chloride form of DEAE Sephadex A-50, and phosphate buffer as eluting agent. The degree of purity was checked by immunoelectrophoresis. Protein concentration was determined by the Lowry method (Lowry *et al.*, 1951).

Purification of thyroglobulin antibody (TgAb). TgAb was purified by affinity chromatography using human thyroglobulin coupled to cyanogen bromide-activated Sepharose 4B, as previously detailed (Pinchera *et al.*, 1977). Serum samples (3 ml) with high TgAb titres were applied to the immunoadsorbent, and the unbound material was removed by washing with borate buffer saline. Purified antibody was eluted by 0·1 M glycine HCl, pH 2·8, neutralized with 0·5 M Na_2HPO_4 and then concentrated by negative pressure dialysis.

Radioiodination procedures. Radioiodination of various IgG preparations and of purified TgAb with ^{125}I was carried out by the lactoperoxidase method, as previously described (Fenzi *et al.*, 1972). Radioiodinated preparations were preadsorbed on human liver homogenates prepared from different subjects in order to remove nonspecifically reacting components.

Cell cultures. Thyroid cell culture was carried out as previously described (Toccafondi *et al.*, 1980a; Toccafondi *et al.*, 1980b). Briefly: normal thyroid tissue obtained at surgery was minced into small fragments, washed in phosphate buffered saline (PBS) and suspended in Krebs-Ringer bicarbonate, pH 7·4, supplemented with trypsin 2·5 g/l (Merck, Darmstadt, West Germany). Trypsinization was continued for 20–30 min at 37°C in 95% O_2-5% CO_2 atmosphere. Thyroid cells were then suspended in McCoy's 5a medium (GIBCO, Grand Island, New York, USA) containing sodium bicarbonate 2·2 g/l and 20% fetal calf serum (GIBCO, Grand Island, New York, USA) in 60 mm Petri culture dishes. Cells cultured at 37°C for 6–8 days were used for TCSAb and TSAb assays. Viability of the cells at the end of the culture period was ascertained according to the following criteria: (a) responsiveness to TSH, as assessed by intracellular cAMP accumulation; (b) release of thyroid hormones and thyroglobulin in the culture medium; (c) persistence of plating efficiency. Twenty-four hours before TCSAb assay culture medium was replaced with McCoy's medium containing 0·4 mM dibutyryl cAMP. On the day of the assay culture medium was substituted with Earle's salt solution containing 2 mM EGTA, the thyroid cells were gently detached and suspended in 0·025 M Tris acetate buffer, pH 7·4, containing 0·25 M sucrose, 1% bovine serum albumin and 10 mM KI. Potassium iodide was added to prevent cellular uptake of ^{125}I possibly deriving from

deiodination of ^{125}I-IgG. Detached thyroid cells were shown to maintain their responsiveness to TSH.

Fibroblasts were obtained from normal volunteers by human skin biopsies on the forearm, cultured as previously described (Rotella *et al.*, 1981) and used as controls.

Studies of binding of radioiodinated IgG to human thyroid cells. Binding studies were carried out as previously described (Toccafondi *et al.*, 1980b) by incubating thyroid cells (approx. 2×10^5 cells) with radioiodinated IgG (approx. 1×10^6 c.p.m.). The final incubation volume was 150 μl. After 30 min incubation at 37°C, the pellet was separated by centrifugation at 800 ***g*** for 5 min at room temperature, washed twice with PBS and then counted. Results were expressed as the percentage of binding observed in absence of unlabelled IgG, subtracted of nonspecific binding (i.e. binding of radioiodinated IgG to cell free tubes). Inhibition of binding was evaluated after addition of graded amounts (0·033–1·7 mg/ml) of unlabelled IgG preparations. Preliminary experiments performed at various time intervals showed that there was no significant change of binding of radioiodinated TCSAb positive IgG per number of cells up to 8 days of culture, indicating persistence of reactive thyroid cell surface autoantigens. For convenience further binding assays were performed after 6–8 days, since the number of cells almost doubled from day 2 to day 8 as assessed by DNA measurements.

Microsomal preparation. Microsomes were prepared by differential centrifugation from specimens of hyman thyroid and liver tissue, as previously described (Mariotti *et al.*, 1979).

Absorption studies. Radioiodinated IgG containing TMAb was incubated for 1 hr at 37°C with either human thyroid microsomes (1·5 mg/ml), human liver microsomes (1·5 mg/ml), human cultured thyroid cells (approx. 9×10^5 cells) or human skin fibroblasts (approx. 9×10^5 cells). The absorbed radioactivity was removed by centrifugation at 143,000 ***g*** for 90 min at 4°C for microsomes or at 800 ***g*** for 5 min at room temperature for cells. The absorption procedure was repeated twice. The final supernatant was tested for binding to thyroid or liver microsomes coated on plastic microtitre plates (Mariotti *et al.*, 1979) and to thyroid cells or fibroblasts. Bound radioactivity was determined and results expressed as percentage of added radioactivity.

Thyroid antibody assays. TSAb was measured by the McKenzie's mouse LATS bioassay (McKenzie, 1958) with minor modifications (Pinchera *et al.*, 1969), and by assaying intracellular cAMP accumulation in human cultured thyroid cells (Toccafondi *et al.*, 1980a). In the latter assay IgG (0·2 mg at a final concentration of 1 mg/ml) was incubated for 60 min at 37°C with cultured human thyroid cells in presence of 0·6 mM 3-isobutyl-1-methylxanthine. The reaction was stopped by the addition of absolute ethanol. The samples were left overnight at −20°C and then centrifuged at 2,000 ***g*** at 4°C. cAMP was measured in the lyophilized supernatant by saturation analysis and the DNA content was determined in the pellet by a fluorometric method (Kissane & Robins, 1958) adapted to thyroid tissue. cAMP accumulation was expressed as pmol/μg DNA. Values greater than 2 s.d. above the mean of results obtained with normal IgG were considered positive for TSAb.

TMAb was measured by competitive binding radioassay (Mariotti *et al.*, 1978), while TgAb was determined by passive haemagglutination (Fulthorpe *et al.*, 1961).

RESULTS

IgG preparations from patients with thyroid autoimmune disorders used in these studies were selected on the basis of results of tests for thyroid antibodies. As indicated in Table 1 three IgG preparations were obtained from patients with Graves' disease: GIgG-1 containing both TSAb and TMAb with undetectable TgAb; GIgG-2 containing only TMAb; GIgG-3 containing only TSAb. Both TMAb and TgAb but not TSAb were present in the IgG preparation deriving from a patient with Hashimoto's thyroiditis (HIgG). Only TMAb was detectable in the IgG preparation from a patient with idiopathic myxoedema (MIgG). Normal IgG (NIgG) with negative tests for thyroid antibodies was used as control.

Binding of radioiodinated IgG to human cultured thyroid cells

The results of binding studies are illustrated in Fig. 1. Significant binding to thyroid cells was observed with all radioiodinated IgG preparations containing TMAb, irrespective of the presence

Table 1. Thyroid antibody pattern of IgG from patients with Graves' disease (GIgG), Hashimoto's thyroiditis (HIgG) and idiopathic myxoedema (MIgG) and from normal subjects (NIgG) used in the binding inhibition studies.

	Thyroid Stimulating Antibody			
	LATS assay‡	TSAb assay§	TMAb*	TgAb†
GIgG-1**	2612	159	486	Neg
GIgG-2	Neg	Neg	12,500	Neg
GIgG-3	300	225	Neg	Neg
HIgG	Neg	Neg	213	1:640
MIgG	Neg	Neg	130	Neg
NIgG	Neg	Neg	Neg	Neg

* Thyroid microsomal antibody (TMAb) was measured by a competitive binding radioassay and results were expressed as u/100 μg IgG.

† Thyroglobulin antibody (TgAb) was measured by passive haemagglutination using IgG (10 mg/ml).

‡ LATS assay was performed by McKenzie's mouse bioassay using 5 mg IgG per mouse and results were expressed as percentage increase of basal blood radioactivity.

§ TSAb was measured by percentage increase of intracellular cAMP accumulation after incubation with IgG (1 mg/ml).

** GIgG-1, GIgG-2 and GIgG-3 refer to IgG preparations from three different patients with Graves' disease.

of TSAb and TgAb; these included GIgG-1, GIgG-2, HIgG and MIgG. Purified TgAb also proved to bind to thyroid cells (0·51%). A negligible binding (<0·1%) was obtained with GIgG-3, which was positive for TSAb, but negative for TMAb or TgAb, and with NIgG. Evidence for the specificity of the binding reaction was provided by the lack of binding (<0·02%) of the various

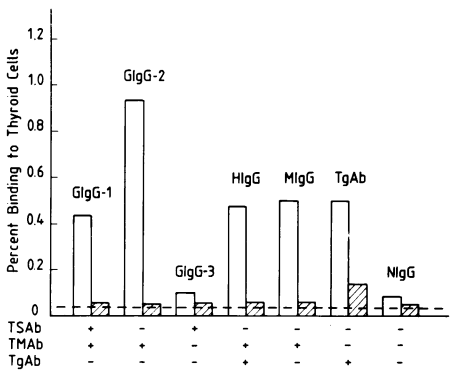

Fig. 1. Binding of different ^{125}I-IgG preparations to human cultured thyroid cells in absence (□) or presence (■) of the same unlabelled IgG (0·67 mg/ml). The dotted area indicates ^{125}I-IgG binding to human fibroblasts. GIgG-1, -2, -3 = Graves' IgG; HIgG = Hashimoto's IgG; MIgG = Idiopathic myxoedema IgG; TgAb = Purified thyroglobulin antibody; NIgG = Normal IgG; TMAb = Thyroid microsomal antibody; TSAb = Thyroid stimulating antibody.

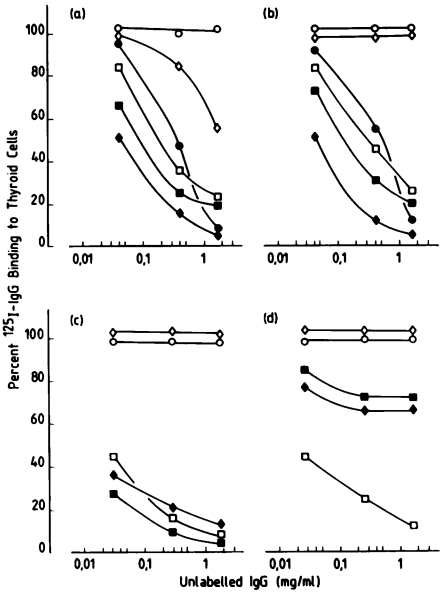

Fig. 2. Effects of graded amounts (0·033–1·7 mg/ml) of different IgG preparations on binding to cultured human thyroid cells of: (a) ^{125}I-GIgG-1; (b) ^{125}I-GIgG-2; (c) ^{125}I-HIgG; (d) ^{125}I-MIgG. Results are expressed as percentage of the binding observed in absence of unlabelled IgG. ● = GIgG-1; ♦ = GIgG-2; ◊ = GIgG-3; ■ = HIgG; □ = MIgG; ○ = NIgG.

radioiodinated IgG preparations to human skin fibroblasts. Furthermore, binding to human thyroid cells could be almost completely inhibited by the addition of an excess of the corresponding unlabelled IgG preparations.

Studies on inhibition of binding of radioiodinated IgG to thyroid cells

In these experiments various unlabelled IgG preparations were tested for their inhibitory activity on binding of each radioiodinated IgG preparation reacting with cultured human thyroid cells. Tests were performed by adding graded amounts of unlabelled IgG. As illustrated in Fig. 2a, a dose-dependent inhibition of binding of ^{125}I-GIgG-1 up to 94% was observed using the corresponding unlabelled IgG preparation, while no effect was produced by NIgG. Strong inhibitory activity was also shown by other TMAb positive IgG preparations (GIgG-2, HIgG, MIgG). However, a definite, although weaker, inhibitory activity was also shown by GIgG-3, which was positive for TSAb, but negative for other antithyroid antibodies. GIgG-3 failed to show direct evidence of binding when tested in the experiments described above, possibly because of a very low concentration of antibodies reacting with thyroid cell surface. Inhibition of binding of ^{125}I-GIgG-2 (Fig. 2b) and of ^{125}I-HIgG (Fig. 2c) was observed with IgG preparations containing TMAb (GIgG-2, HIgG and MIgG), but not with TMAb negative IgG (GIgG-3 and NIgG). As shown in Fig. 2d, a dose-dependent inhibition (up to 90%) of ^{125}I-MIgG binding to thyroid cells was produced by unlabelled MIgG. GIgG-2 and HIgG produced an inhibition of 35% and 31%, respectively, when tested at a concentration of 0·7 mg/ml, and no further inhibition could be obtained when the concentration of these IgG preparations was increased to 1·7 mg/ml. No effect was observed with GIgG-3 or NIgG.

Absorption experiments

The binding of radioiodinated thyroid microsomal IgG (i.e. IgG containing TMAb, but not TgAb or TSAb) to human thyroid microsomes and cultured human thyroid cells was evaluated before and after preabsorption with thyroid microsomes or thyroid cells. Human liver microsomes and human skin fibroblasts were used as control. The results of these experiments are reported in Table 2.

Table 2. Effects of preabsorption with human thyroid microsomes (TMC), human liver microsomes (LMC), cultured human thyroid cells (TC) or cultured human fibroblasts (FB) on binding of radioiodinated thyroid microsomal IgG to the same material.

Materials used for preabsorption	% Binding			
	TMC	LMC	TC	FB
None	3·38	0·06	1·28	0·04
TMC	0	0	0·01	0
LMC	3·10	0	0·95	0
TC	1·78	0	0·27	0
FB	3·36	0	0·95	0

This IgG preparation was shown to bind to thyroid microsomes (3·38%) and to thyroid cells (1·28%), but not to liver microsomes and skin fibroblasts. Preabsorption with thyroid microsomes resulted in a complete removal of binding activity to both thyroid microsomes and thyroid cells. Preabsorption with thyroid cells produced a 79% reduction of binding to thyroid cells, while a reduction of only 48% was observed in the binding to thyroid microsomes. Preabsorption with liver microsomes or skin fibroblasts only slightly affected subsequent binding to thyroid microsomes or thyroid cells.

DISCUSSION

The present report shows that radioiodinated immunoglobulins from patients with thyroid autoimmune disorders are able to bind to viable thyroid cells, indicating a reaction with thyroid cell surface antigens. This is in agreement with similar observations obtained by immunofluorescence (Fagraeus & Jonsson, 1970; Khoury *et al.*, 1980) and mixed haemadsorption (Jonsson & Fagraeus, 1969) techniques. Evidence for tissue specificity was provided by the absence of binding of these radioimmunoglobulins to human skin fibroblasts. The binding cannot be explained by a non-specific reactivity of immunoglobulins, since radioiodinated IgG from normal subjects did not bind to cultured thyroid cells. Furthermore, addition of the original unlabelled IgG preparations results in a dose-dependent inhibition of thyroid binding of radioimmunoglobulins from patients with thyroid autoimmune disorders, while normal IgG was ineffective. Assays for TCSAb were performed using human thyroid cells cultured for 6–8 days. Binding experiments showed that no loss of reactive surface antigens occurred during this period of time, as assessed by binding experiments. A decline of thyroid cell surface antigens after 1 week of culture, as assessed by immunofluorescence or cytotoxicity techniques, and by mixed haemadsorption has been reported by other workers (Forbes *et al.*, 1962; Kite *et al.*, 1965; Nicol & Swanson-Beck, 1966; Jonsson, Fagraeus & Biberfield, 1968). The discrepancy possibly referred to a difference in the sensitivity of the methods used.

A significant correlation between TCSAb and TMAb in sera of patients with thyroid autoimmune disorders has been previously documented in our (Pinchera *et al.*, 1980b) and others' laboratories (Khoury *et al.*, 1980). Consistent with this finding are the present data, indicating that binding to thyroid cells could be observed with radioiodinated IgG preparations from patients with Graves' disease, Hashimoto's thyroiditis or idiopathic myxoedema containing elevated levels of TMAb, but not with a Graves' IgG preparation with undetectable TMAb. These results suggest that the microsomal antigen is expressed on the thyroid cell surface. Previous experiments performed with thyroid subcellular fractions demonstrated that plasma membrane antigens are commonly present in microsomal preparations (Pinchera *et al.*, 1976a; Pinchera *et al.*, 1976b). Since

the microsomal antigen has been shown to be a lipoprotein component of exocytotic vesicles (Roitt *et al.*, 1964), which contribute to the membrane material of the apical cell surface (Ekholm *et al.*, 1975), the presence of common antigenic determinants in thyroid microsomes and on the thyroid cell surface should not be surprising.

To further investigate the relationship between thyroid cell surface antigens and thyroid microsomal antigen, the binding to thyroid cells and thyroid microsomes of radioiodinated IgG containing TMAb was studied before and after preabsorption with thyroid microsomes or thyroid cells. Binding to both thyroid microsomes and thyroid cells could be completely abolished by preabsorption with thyroid microsomes, indicating that all thyroid cell surface antigens are also present in the microsomal fraction. Preabsorption with thyroid cells was much more effective on binding to thyroid cells than on binding to thyroid microsomes, suggesting that not all microsomal antigens are expressed on the thyroid cell surface. However, it is conceivable that the antigen expressed on the surface of thyroid cells is much less concentrated than that contained in the microsomal fraction. Thus, it cannot be excluded that the incomplete removal of microsomal reactivity could be explained by the use of an insufficient number of cells or of a relatively short absorption time.

Experiments performed with radioiodinated Graves' IgG positive for TSAb but negative for TMAb and TgAb failed to provide evidence of binding of TSAb to cultured human thyroid cells, confirming previous studies on thyroid plasma membrane fractions (Pinchera *et al.*, 1976a). This finding does not necessarily exclude binding of TSAb to the cell surface, since the concentration of TSAb in the IgG fraction could be too low to be detected in the present assay. It has been recently proposed that the absence of binding of radioiodinated TSAb could be due to the loss of biological activity produced by the radioiodination procedure (Mehdi & Kriss, 1978). To circumvent this problem Mehdi & Kriss (1978) recombined TSAb heavy chains with ^{125}I-Bence–Jones light chains and found binding of this product to human thyroid membranes. They also reported that a similar produce obtained with ^{125}I-Bence–Jones light chains regained full biological activity. The significance of this finding is uncertain, since, at variance with these authors, Zakarija and McKenzie (1980) were unable to show restoration of biological activity by recombining TSAb heavy chain with unrelated light chain. Moreover, the enzymic iodination used in the present study has been previously shown to produce no adverse effect on TSAb biological activity (Fenzi *et al.*, 1972).

Purified radioiodinated TgAb showed specific binding to thyroid cells, indicating the presence of thyroglobulin antigenic determinants on the cell surface. Specific receptors for thyroglobulin on thyroid plasma membranes have been recently described (Consiglio *et al.*, 1979). Since cultured thyroid cells have been shown to release thyroglobulin in the medium (unpublished data), it is conceivable that some of the secreted molecules of the protein may bind to the surface receptors. Further evidence of thyroglobulin interaction with cell surface components is provided by the observation that this protein inhibits TSH binding to thyroid plasma membranes (Hashizume, Fenzi & DeGroot, 1979) and reduces the TSH-induced cAMP intracellular accumulation in cultured thyroid cells (Aterini *et al.*, 1980).

Unlabelled IgG containing TMAb and deriving from patients with thyroid autoimmune disorders produced a dose-dependent inhibition of the binding to thyroid cells of TMAb positive radioiodinated IgG obtained from different subjects with either Graves' disease, Hashimoto's thyroiditis or idiopathic myxoedema. This indicates that common antigen–antibody systems are involved in these diseases. However, some inconsistencies with this concept were observed. A Graves' IgG preparation with detectable TSAb and undetectable TMAb failed to inhibit the binding of other three radioiodinated IgG preparations positive for TMAb, but negative for TSAb, but produced a partial inhibition of the binding of a radioiodinated Graves' IgG preparation with both positive TSAb and TMAb assays. These data might suggest that TSAb is responsible for the inhibitory effect, but this interpretation is not consistent with the finding that TSAb negative IgG preparations from patients with either Hasimoto's thyroiditis or idiopathic myxoedema produced an almost complete inhibition in the same system. The presence of anti-A or anti-B isohaemagglutinins in both the radioiodinated and the unlabelled IgG preparations may result in a false positive reaction when blood group A or B cultured human thyroid cells are used for the detection of thyroid cell surface antibodies (Khoury *et al.*, 1981). This possibility can be ruled out, since blood group O

thyroid cells were used in the present experiments. Interestingly, the binding of radioiodinated IgG from a TMAb positive patient with idiopathic myxoedema was virtually completely inhibited by the original unlabelled IgG, but not by two IgG preparations with elevated TMAb levels deriving from patients with either Graves' disease or Hashimoto's thyroiditis. Thus, it would appear that some of the thyroid cell surface antibodies found in this patient differed from those present in the patients with other thyroid autoimmune disorders. The question of whether this also occurs in other patients with idiopathic myxoedema remains to be elucidated. Clearly these preliminary observations need to be confirmed and extended in a larger series of sera.

In conclusion, the present results have provided further support to the concept that thyroid microsomal antibody is able to recognize certain antigens represented on the surface of human thyroid cells in culture and suggest that individual thyroid autoimmune sera may possess a variety of specificities, some of which have not yet been identified. However, the use of a rather limited number of sera selected from a heterogeneous thyroid autoimmune population calls for caution in the interpretation of the results.

REFERENCES

ATERINI, S., ROTELLA, C.M., TANINI, A., ZONEFRATI, R. & TOCCAFONDI, R.S. (1980) Inhibition of thyrotropin-stimulated cyclic AMP accumulation by human thyroglobulin in human cultured thyroid cells. *FEBS Lett.* **121,** 44.

BAUMSTARK, J.S., LAFFIN, R.J. & BARDAWILL, W.A. (1964) A preparative method for the separation of 7S gamma globulin from human serum. *Arch. Biochem. Biophys.* **108,** 514.

CONSIGLIO, E., SALVATORE, G., RALL, J.E. & KOHN, L.D. (1979) Thyroglobulin interactions with thyroid plasma membranes. *J. Biol. Chem.* **254,** 5065.

EKHOLM, R., ENGSTRÖM, G., ERICSON, L.E. & MELANDER, A. (1975) Exocytosis of protein into the thyroid follicle lumen: an early effect of TSH. *Endocrinology*, **97,** 337.

FAGRAEUS, A. & JONSSON, J. (1970) Distribution of organ antigens over the surface of thyroid cells as examined by the immunofluorescence test. *Immunology*, **18,** 413.

FENZI, G.F., MACCHIA, E., BARTALENA, L., MAZZANTI, F., BASCHIERI, L. & DEGROOT, L.J. (1978) Radioreceptor assay of TSH: its use to detect thyroid-stimulating immunoglobulins. *J. Endocrinol. Invest.* **1,** 17.

FENZI, G.F., ROMALDINI, J.H., GRASSO, L., RACCIS, E., PINCHERA, A. & BASCHIERI, L. (1972) Effects of various iodination procedures on the biological activity of LATS. *J. Nucl. Med. All. Sci.* **16,** 55.

FORBES, I.J., ROITT, I.M., DONIACH, D. & SOLOMON, I.L. (1962) The thyroid cytotoxic autoantibody. *J. clin. Invest.* **41,** 996.

FULTHORPE, A.J., ROITT, I.M., DONIACH, D. & COUCHMANN, K. (1961) A stable sheep-cell preparation for detecting thyroglobulin autoantibodies and its clinical application. *J. clin. Path.* **14,** 654.

HASHIZUME, K., FENZI, G.F. & DEGROOT, L.J. (1979) Thyroglobulin inhibition of thyrotropin binding to thyroid plasma membrane. *J. clin. Endocrinol. Metab.* **46,** 679.

JONSSON, J. & FAGRAEUS, A. (1969) On the mechanism of the ring zone effect obtained with the mixed haemadsorption technique. *Immunology*, **17,** 387.

JONSSON, J., FAGRAEUS, A. & BIBERFELD, G. (1968) The mixed haemadsorption test as an aid to the diagnosis of thyroid autoimmune disease. *Clin. exp. Immunol.* **3,** 287.

KHOURY, E.L., HAMMOND, L.J., BOTTAZZO, G.F. & DONIACH, D. (1980) Detection of antibodies to cell surface by immunofluorescence on human thyroid cultures. In *Autoimmune Aspects of Endocrine Disorders* (ed. by A. Pinchera, D. Doniach, G. F. Fenzi & L. Baschieri), p. 163. Academic Press, London.

KHOURY, E.L., HAMMOND, L., BOTTAZZO, G.F. & DONIACH, D. (1981) Presence of the organ-specific 'microsomal' autoantigen on the surface of human thyroid cells in culture: its involvement in complement-mediated cytotoxicity. *Clin. exp. Immunol.* **45,** 316.

KISSANE, J.M. & ROBINS, E. (1958) The fluorometric measurement of deoxyribonucleic acid in animal tissue with special reference to the central nervous system. *J. Biol. Chem.* **233,** 184.

KITE, J.H., JR., ROSE, N.R., KANO, K. & WITEBSKY, E. (1965) Cytotoxicity of human thyroid autoantibodies. *Ann. N.Y. Acad. Sci.* **124,** 626.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265.

MARIOTTI, S., PINCHERA, A., VITTI, P., CHIOVATO, L., MARCOCCI, C., URBANO, C., TOSI, M. & BASCHIERI, L. (1978) Comparison of radioassay and haemagglutination methods for anti-thyroid microsomal antibodies. *Clin. exp. Immunol.* **34,** 118.

MARIOTTI, S., PINCHERA, A., MARCOCCI, C., VITTI, P., URBANO, C., CHIOVATO, L., TOSI, M. & BASCHIERI, L. (1979) Solubilization of human thyroid microsomal antigen. *J. clin. Endocrinol. Metab.* **48,** 207.

MCKENZIE, J.M. (1958) The bioassay of thyrotropin in serum. *Endocrinology*, **63,** 372.

MEHDI, S.Q. & KRISS, J.P. (1978) Preparation of radiolabeled thyroid-stimulating immunoglobulins (TSI) by recombining TSI heavy chains with ^{125}I-labeled light chains: direct evidence that the product binds to the membrane thyrotropin receptor and stimulates adenylate cyclase. *Endocrinology*, **103,** 296.

NICOL, A.G. & SWANSON-BECK, J. (1966) Persistence

of an organ-specific antigen in organ and tissue cultures of hyperplastic human thyroid gland. *Nature*, **210**, 1227.

PINCHERA, A., LIBERTI, P., MARTINO, E., FENZI, G.F., GRASSO, L., ROVIS, L., BASCHIERI, L. & DORIA, G. (1969) Effects of antithyroid therapy on the long acting thyroid stimulator and the antithyroglobulin antibodies. *J. clin. Endocrinol. Metab.* **29**, 231.

PINCHERA, A., MARIOTTI, S., VITTI, P., FENZI, G.F., GRASSO, L., TOSI, M. & BINDI, S. (1976a) Studies on thyroid plasma membrane antigens. In *Biochemical Basis of Thyroid Stimulation and Thyroid Hormone Action* (ed. by A. Von zür Muhlen & H. Schleusener), p. 79, Georg Thieme Publ., Stuttgart.

PINCHERA, A., MARIOTTI, S., VITTI, P., GRASSO, L., PACINI, F., BASCHIERI, L. & STANBURY, J.B. (1976b) Studies on the nature of thyroid antigens involved in thyroid autoimmune disorders. In *Thyroid Research* (ed. by J. Robbins & L. E. Braverman), p. 395. Excerpta Medica, Amsterdam.

PINCHERA, A., MARIOTTI, S., VITTI, P., TOSI, M., GRASSO, L., PACINI, F., BUTI, R. & BASCHIERI, L. (1977) Interference of serum thyroglobulin in the radioassay for serum antithyroglobulin antibodies. *J. clin. Endocrinol. Metab.* **45**, 1077.

PINCHERA, A., FENZI, G.F., BARTALENA, L., CHIOVATO, L., MARCOCCI, C. & BASCHIERI, L. (1980a) Antigen–antibody systems involved in thyroid autoimmunity. In *Autoimmune Aspects of Endocrine Disorder* (ed. by A. Pinchera, D. Doniach, G. F. Fenzi & L. Baschieri). p. 67. Academic Press, London.

PINCHERA, A., FENZI, G.F., BARTALENA, L., CHIOVATO, L., MARCOCCI, C., TOCCAFONDI, R., ROTELLA, C.M., ATERINI, S. & ZONEFRATI, R. (1980b) Thyroid cell surface and thyroid stimulating antibodies in patients with thyroid autoimmune disorders. In *Thyroid Research VIII* (ed. by J. R. Stockigt & S. Nagataki), p. 707. Australian Academy of Science, Canberra.

PINCHERA, A., FENZI, G.F., VITTI, P., MACCHIA, E., TOCCAFONDI, R. & BASCHIERI, L. (1980c) Relationship between TSH receptor and thyroid plasma membrane antigens for Graves' disease immunoglobulins. In *Endocrinology 1980* (ed. by I. A. Cumming, J. W. Funder & F. A. O. Mendelsohn), p. 126. Australian Academy of Science, Canberra.

ROITT, I.M., LING, N.R., DONIACH, D. & COUCHMANN, K.G. (1964) The cytoplasmatic autoantigen of the human thyroid. I. Immunological and biochemical characteristics. *Immunology*, **7**, 375.

ROTELLA, C.M., ZONEFRATI, R., TOCCAFONDI, R. & D'ALESSANDRO, A. (1981) Reduced mytogenic action of insulin evaluated by ^{3}H-thymidine uptake in diabetic cultured fibroblasts. *Horm. Metab. Res.* in press.

TOCCAFONDI, R.S., ATERINI, S., MEDICI, M.A., ROTELLA, C.M., TANINI, A. & ZONEFRATI, R. (1980a) Thyroid-stimulating antibody (TSAb) detected in sera of Graves' patients using human thyroid cell cultures. *Clin. exp. Immunol.* **40**, 532.

TOCCAFONDI, R.S., ROTELLA, C.M., MEDICI, M.A., BARTALENA, L., CHIOVATO, L., MARCOCCI, C., FENZI, G.F. & PINCHERA, A. (1980b) Binding of radioiodinated autoantibodies to intact human thyroid cells: evidence for cell surface antigens. In *Autoimmune Aspects of Endocrine Disorders* (ed. by A. Pinchera, D. Doniach, G. F. Fenzi & L. Baschieri), p. 157. Academic Press, London.

ZAKARIJA, M. & MCKENZIE, J.M. (1980) Immunochemical characterization of TSAb: evidence against polyclonal nature. In *Thyroid Research VIII* (ed. by J. R. Stockigt & S. Nagataki), p. 669. Australian Academy of Science, Canberra.