Journal of Cellular Biochemistry 31:107–120 (1986)

The Thyroid "Microsomal" Antigen Is an Epitope on the Thyrotropin Receptor

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Antimicrosomal antibodies are present in the sera of most patients with autoimmune thyroiditis, and Graves' disease. It has, in general, been difficult to separate antimicrosomal activity from that directed against the thyrotropin (TSH) receptor in Graves' IgG preparations. The "microsomal" antigen has been localized to the endoplasmic reticulum and microfollicular aspect of thyrocytes; its structure is however unknown. In an attempt to identify the thyroid microsomal antigen, we studied the interaction of Hashimoto's IgG with high microsomal antibody titre and negative for thyroglobulin with purified thyroid plasma and light microsomal membranes. We allowed Hashimoto's, Graves', and control IgGs to bind to protein blots of thyroid plasma membranes resolved on SDS-PAGE under non-reducing conditions. All seven Hashimoto's IgG at a concentration of 2 mg/ml interacted with an M ~ 197,000 polypeptide corresponding to the TSH holoreceptor. By contrast to Graves' IgG (which were positive at 1 mg/ml), however, this binding was not blocked by pretreatment of the protein blots with TSH. Normal IgGs showed no binding at concentrations of up to 2 mg/ml.

Both Hashimoto's and Graves' IgG interacted with TSH-affinity column-purified receptor preparations.

Two of the Hashimoto's IgGs induced adenylate cyclase activation in thyroid plasma membranes, three inhibited TSH-stimulated enzyme activation, and two were without effect. Two classes of autoantibodies, other than TSH receptor directed, were encountered; one class raised to antigens common to all seven patients and another class unique to individual patients, eg, M_r 210,000 and M_r 20,000 polypeptides.

We propose that the TSH receptor has multiple epitopes (functional domains), and the one to which antimicrosomal antibody bind is likely to be spatially separated from that with which Graves' IgG and TSH interact. Differences in affinity or number of sites allows for the demonstration of Graves' IgG against a background of antimicrosomal antibody.

Key words: Hashimoto's thyroiditis, Graves' disease, microsomal antigen, TSH receptor

Received April 10, 1985; revised and accepted October 28, 1985.

Recently, progress has been made in elucidating the genetic basis as well as the associated immunoregulatory defects of human autoimmune endocrine disorders (see [1] for review). By contrast, much less is known about the antigens against which cell-mediated and humoral autoimmunity are directed. Thyroid epithelial cell antigens have been studied the longest [2]. It is generally accepted that the IgG antibody which interacts with the thyrotropin (TSH) receptor to stimulate thyroid follicular cells is highly specific for Graves' disease [3-5] and is found in only few instances in autoimmune thyroiditis [5]. Some reports have, however, suggested that as many as 50% of patients with autoimmune goitrous thyroiditis have circulating antibodies capable of binding to the TSH receptor and inducing adenylate cyclase activation [6,7].

Using protein blotting technique [8], we now show that sera from patients with autoimmune thyroiditis interact with the TSH holoreceptor apparently at antigenic sites separate from those for TSH and Graves' IgG [9]. We have also identified a number of autoantigens shared between thyroid plasma membranes and microsomes.

MATERIALS AND METHODS

Subjects

We studied IgG from seven consecutive patients with autoimmune thyroiditis who had antimicrosomal antibody titres of $\geqslant$ 1/1600 but no antithyroglobulin antibodies by haemmaglutination. Five had goitres. The IgG from ten healthy individuals negative for thyroid antibodies were used as controls.

Preparation of Thyroid Subcellular Fractions

Porcine thyroids were finely sliced, and minced tissue was briefly homogenized with 10 volumes of 10 mM Tris-HCl, pH 7.4, containing 0.25 M sucrose, 1 mM MgC1, and 1 mM EGTA (STEM), using the low speed of a polytron (Brinkmann Instruments, Westbury, NY). The cell pellets obtained from centrifuging the homogenate at 7,800g for 10 min were resuspended in 10 volumes of STEM and filtered through nylon mesh. The cells were then disrupted by nitrogen decompression at 950 p.s.i. using PARR cell bomb (PARR Instrument Company, Moline, IL). The homogenate was centrifuged at 1,000g for 10 min and the supernatant further centrifuged at 4,500g for 10 min. Crude membranes were collected from the supernatant by centrifugation at 103,000g for 30 min. The pelleted membranes were suspended in 55% sucrose in 10 mM Tris-HCl, pH 7.4, containing 1 mM $MgCl_2$ and 1 mM EGTA (TEM) by homogenization and placed in 3-ml volumes into cellulose nitrate centrifuge tubes. Sucrose solutions (3.5 ml each) of 45%, 40%, 35%, and 30%, respectively, were carefully layered over the membrane suspension. After centrifugation at 103,000g for 90 min using a Beckman Sw-27 rotor, the bands at the 35%-40% (light microsomes) and at the 40%-45% (plasma membranes) gradient interfaces were collected, diluted with TEM to a final sucrose concentration of 0.25 M, and again centrifuged at 103,000g for 30 min. The pellet was finally suspended in a 0.5 ml STEM, and the protein content [10] was adjusted to 2 mg/ml.

The light microsomal and plasma membrane fractions were assayed for 5′-nucleotidase and NADPH cytochrome C reductase to determine the degree of their purity. The 5′-nucleotidase activity (in mole Pi/hr/mg protein) was 6.0 in crude thyroid tissue homogenates, 12 in microsomal fraction, and 40.8 in the plasma

membrane fraction. By contrast NADPH cytochrome C reductase activity (as ΔOD_{550}/min/mg protein) was 0.125 in crude homogenates and 0.666 and 0.20, respectively, in microsomal and plasma membrane fractions.

Affinity Purification of the TSH Receptor

The cross-linking of bTSH to Affigel-10 (Bio-rad, Richmond, CA) was previously described [11]. The TSH-Affigel conjugate was washed with 3 M NaCl and equilibrated with 40 mM Tris-HCl, pH 7.4. Five millitres of the conjugate were packed into 0.9- × 15- cm column and incubated with 1.5 mg of peak VIII proteins for 16 hr at 4°C. The column was then washed with 40 mM Tris-HCl, pH 7.4, until absorbance (at 280 nm) dropped to baseline; proteins bound to the column were eluted with the same buffer containing 3 M NaCl. The eluates were desalted by dialysis against 50 mM Tris-HCl/2 M sucrose, pH 7.4, and sucrose concentration subsequently reduced to 0.25 M.

Interaction of Microsomal and Plasma Membrane Polypeptides With IgG

One hundred micrograms of thyroid plasma, light microsomal membranes, and porcine thyroglobulin (Sigma, St. Louis, MO) were resolved on 7.5%-15.0% linear gradient polyacrylamide slab gel containing 1% sodium dodecyl sulphate (SDS) [12] in the presence or absence of 5% mercaptoethanol. The peptide bands thus resolved were electrotransferred to nitrocellulose paper (Schleicher and Schuell Inc., Keene, NH) overnight at 200 mA using a Hoffer Scientific Instrument TE 42 Transphor-electrophoretic transfer unit. Individual lanes were cut and incubated with 1 U/ml bTSH for 5 hr or 2 mg/ml IgG (and anti-TSH in the case of TSH) for 14 hr at 4°C. Graves' IgG was used at a concentration of 1 mg/ml [9]. In some experiments, IgG from a high titre rabbit serum raised against human thyroglobulin but which completely cross-reacts with porcine thyroglobulin (a gift from Dr. Ian R. Senciall, Faculty of Medicine, Memorial University) was used as positive control at a concentration of 1 mg/ml. At the end of incubation, the nitrocellulose paper strips were washed several times with 50 mM Tris-HCl, 200 mM NaCl, pH 7.4 (TBS), to remove excess antibody and peroxidase-conjugated species-specific anti-IgG (Cappel Laboratories, Cochranville, PA) diluted 1/500 in TBS was added to each strip and incubated for 4 hr at room temperature. The unbound antibody was then removed by washing with several changes of TBS, and the paper was developed with 4-chloro-1-naphthol (Sigma, St. Louis, MO) and hydrogen peroxide for 15 min [9].

Interaction of Hashimoto's and Graves' IgG With Affinity-Purified Receptor

Five- and 10-ug samples of affinity-purified-receptor preparation were dotted on nitrocellulose paper (Millipore Ltd., Mississauga, Ontario), which had been divided into 6 × 6 mm grids. The dots were dried under nitrogen, and the paper was washed with 50 mM Tris-HCl, 200 mM NaCl, pH 7.4 (TBS). While still wet, the nitrocellulose paper was cut into individual squares and placed into the well of a 24 Well Costar tray (Cambridge, MA). The unreacted sites were blocked with a mixture of 10% horse serum and 3% bovine serum albumin in TBS. After washing, the paper squares were incubated with 300 μl Hashimoto's IgG (2 mg/ml), Graves', and normal human IgGs (1 mg/ml) for 14-16 hr at 4°C. The papers were further treated to visualize bound antibodies as outlined above for SDS-PAGE resolved membrane preparations.

Stimulation of Thyroid Adenylate Cyclase (AC) Activity

The effects of 10 to 200 mU/ml of bTSH, 250 ug/ml of normal, or Hashimoto's patients' IgG on AC activity of porcine thyroid plasma membrane was studied over a period of 20 min. The influence of these IgGs upon TSH (100 mU/ml)-stimulated AC was also studied. AC was assayed in a final volume of 20 ul in 50 mM Tris-HCl, pH 7.5, for 20 min at 37°C in the presence of 10 uM guanosine-5-(β-α-imide) triphosphate [13]. Reaction was always initiated by adding equal volumes of ATP-regenerating system containing—4 mM Mg^{2+}, 5 mM theophyllin, 2 mM ATP, 15 mM creatine phosphate, and 25 μg rabbit muscle creatine phosphokinase to 40 μg of plasma membranes [14]. The cAMP was measured by a protein-binding assay [15]. An increase of basal or decrease of TSH-stimulated AC activity by $\geqslant$ 30% compared to normal IgG were the criteria for sera influencing this system.

Production of Monoclonal Antibodies Against Thyroid Antigens

BALB/c mice received a subcutaneous injection of 100 ug thyroid membrane prepared as above in complete Freund's adjuvant and, on days 8 and 28, two 50-μg injections of incomplete adjuvant. Three days after the last injection, spleens were removed, dissociated mechanically and 1×10^8 spleen cells were fused with 2×10^7 Sp2/0-Ag16 non-secreting mouse myeloma cells (provided by Dr. Louise Lefleur, Cancer Research Center, Quebec City) in the presence of polyethylene glycol, M.W. 4,000 [16]. After fusion, cells were incubated in the presence of hypoxanthine-aminopterin-thymidine medium at 37°C in a humified 95%/5% air/CO_2 atmosphere.

Wells which contained visible colonies (10-14 days after fusion) were assayed for the presence of anti-thyroid membrane antibodies by a previously described dot immunoblotting assay [9]. The selected hybrid lines were then subcloned four times and expanded in tissue culture in selective medium. The supernatants from these clones were tested for their ability to interact with thyroid plasma membrane polypeptide bands as outlined above. Seven clones were found to be specific for polypeptides of relative molecular weight (M_r) of approximately 210,000 polypeptide (pp 210). Monoclonal TR 17.193 was selected for further studies. Monoclonal immunoglobulin isotype and subclass were ascertained with specific anti-mouse antisera (Meloy, Springfield, IL) by immunodiffusion.

RESULTS

All seven samples of Hashimoto's disease IgG tested interacted with an M_r 197,000 on protein blots of thyroid plasma membranes resolved under non-reducing conditions (Fig. 1a). This polypeptide band represents the TSH holoreceptor [9,11] (Fig. 1b). Ten control sera tested at IgG concentrations of 1, 1.5, and 2 mg/ml were, by contrast, negative (seven are shown in Fig. 2). As there was no difference in the patterns obtained with the three concentrations, only the results with 1.5 mg/ml are shown for seven patients.

The interaction of Graves' IgG with the M_r 197,000 band was blocked by preincubation of the protein blots with native bTSH (Fig. 1a) [9]. Preincubation of similar blots with bTSH was without effect on the binding of Hashimoto's IgG to the TSH holoreceptor band. This result suggested that Hashimoto's IgG bind to domains on the TSH receptor separate from those to which TSH and Graves' IgG bind.

In an attempt to investigate the nature of the antigen(s) of the thyroid microsomal autoantibodies and to ascertain whether these antigens are shared with plasma mem-

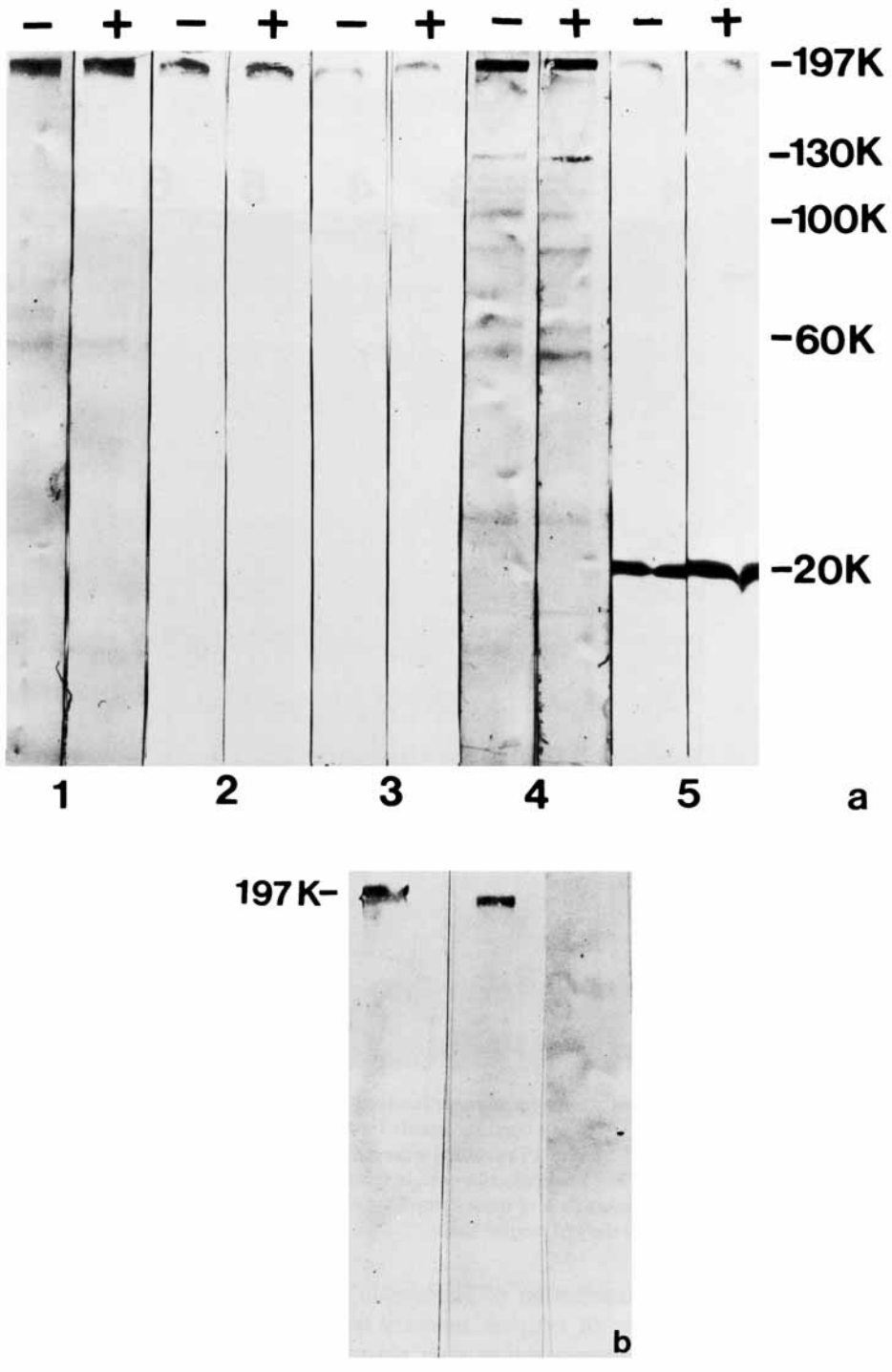

Fig. 1. a) The interaction of Hashimoto's IgG with thyroid plasma membrane peptides. These were resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis in the absence of reductant and transferred to nitrocellulose paper. Binding of Hashimoto's IgG was tested in the presence (+) or absence (−) of native TSH. Samples from patients 3 and 5 stimulated AC; those from patients 1 and 2 inhibited TSH-stimulated AC, whereas IgG from patient 4 had no effect on AC activity. Note that sample 4 has a distinct band larger than TSH receptor shown as 197K and that sample 5 has a unique 20K polypeptide (K = kilodaltons). Molecular weight references are myosin (M_r = 210,000), phosphorylase b (M_r = 94,000), bovine serum albumin (M_r = 67,000), ovalbumin (M_r = 43,000), carbonic anhydrase (M_r = 30,000), soybean trypsin inhibitor (M_r = 20,000) and α-lactalbumin (M_r = 14,000). b) The 197K holoreceptor band interacts with bTSH (Lane 1) and Graves' IgG (Lane 2); the interaction of Graves' IgG with the receptor is inhibited by preincubation with native bTSH (Lane 3).

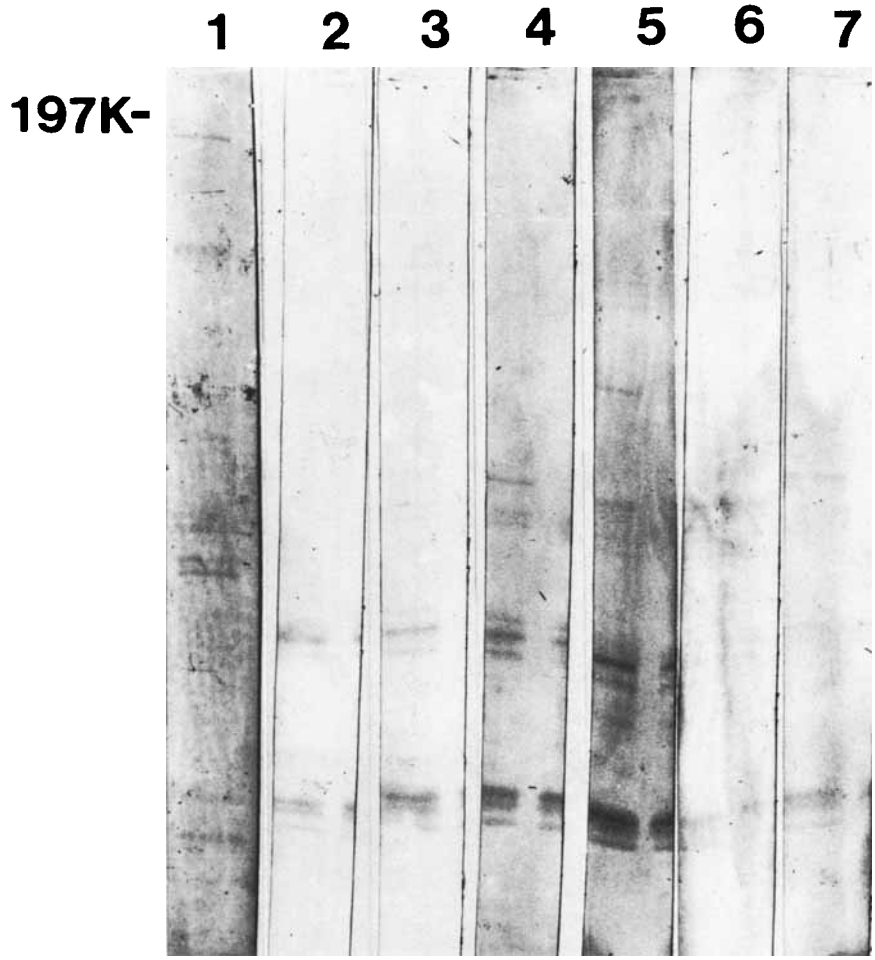

Fig. 2. Protein blots of thyroid plasma membranes resolved on SDS-PAGE in the absence of reductant and allowed to interact with six of ten controls tested. Interaction of control IgG were examined at concentrations of 1.0, 1.5, and 2 mg/ml. The results were indistinguishable. We show here blots reacted with 1.5 mg/ml control IgG. Only low-molecular-weight bands are visualized; the nature of these bands are unknown. The pattern of interaction of these control IgGs with blots of plasma membranes resolved under reducing conditions also showed similar bands.

branes, we studied the interaction of Hashimoto's IgG with light microsomes (see Figs. 1 and 4). Analysis of enzyme markers makes it unlikely that the result is accounted for by cross-contamination with plasma membranes. The thyroid membranes share a large number of antigens with plasma membrane including the TSH receptor (Figs. 1 and 4).

In addition to the TSH receptor, Hashimoto's IgGs show two interaction patterns. The first pattern common to sera tested entailed binding with variable degrees of intensity to several polypeptides over a wide molecular weight range. The second pattern was distinctive for individual sera. One IgG preparation strongly bound to an M_r 210,000 polypeptide (pp 210), whereas another IgG preparation interacted with a

small polypeptide (M_r ~ 20,000) (Fig. 1, 4 lower panel). Polypeptides associated with each of the two patterns were more abundant in plasma membrane than in microsomal preparations. The two "unique" polypeptides were apparently not made of subunits, although their electrophoretic mobilities were enhanced in the presence of reductant (Figs. 2 and 3, upper panel). The M_r 210,000 is now seen to migrate to a distance consistent with M_r 180,000, whereas the M_r 20,000 is reduced to M_r 12,500. Interestingly, IgGs not demonstrating a discernible band at M_r 210,000 under non-reducing conditions are seen to have faint but distinct bands at the M_r 180,000 under reducing conditions (Figs. 3 and 4). Other electrophoretic bands, eg, M_r ~28,500 (lane 1, Fig. 3) were only visualized following reduction and was presumably derived from heavier complexes. The two patients without goitre exhibited the "common" pattern.

There was a notable absence of M_r 197,000 band in protein blots of plasma and microsomal membranes resolved under reducing conditions (Figs. 3 and 4). This finding emphasizes the essential role of intact disulphide bridges for Hashimoto's IgG binding, as has been previously demonstrated for TSH and Graves' IgG [5,9].

The interaction of Hashimoto's IgG with affinity-purified TSH receptor dotted onto nitrocellulose paper excludes the possibility that the antibody bound to a polypeptide of a molecular weight similar to that of the receptor (Fig. 5). We have previously [11] found that affinity purified receptor preparations were made up of holoreceptor ($\leqslant$ 70%) and breakdown products.

The monoclonal antibody raised as a result of immunizing with purified thyroid plasma membrane and selected for further study was found to be specific to M_r 210,000, polypeptide. This band (Fig. 6) is identical to that with serum 4 (Fig. 1). This monoclonal was without effect on ^{125}I-bTSH binding to thyroid plasma membrane or in inducing adenylate cyclase. This antibody is, indeed, specific for thyroglobulin (see below); its further fine specificity will be reported separately.The monoclonal is an IgG_1.

Next, in view of the abundance of thyroglobulin in thyroid epithelial cell membranes [17], we wanted to find whether some of the antigens identified were related to thyroglobulin. Although porcine thyroglobulin is composed of two 330,000 covalently linked subunits and would therefore be expected not to enter the gel, the methods used for the preparation of commercial thyroglobulin as well as to obtain thyroid cellular membrane encourage thyroglobulin degradation [18]. Several Hashimoto's IgG specimens were found to interact more or less strongly with M_r 210,000 and 180,000 bands under both reducing (not shown) and non-reducing conditions (Fig. 7). Monoclonal TR 17.193, also, binds strongly to both these bands suggesting that they both share the same epitope. The bands are unrelated to the TSH receptor with which Graves' IgG bind (Fig. 7). That membrane related M_r 210,000 polypeptide (Figs. 1, 3, and 4) changes its mobility to M_r 180,000 band in the presence of reductant suggests that the smaller peptide is derived from M_r 210,000.

It is clear that the patterns of interaction of rabbit anti-thyroglobulin bears similarities to that of plasma and microsomal membranes resolved under reducing conditions and allowed to bind to Hashimoto's IgG (Figs. 3 and 4, upper panel). Identity of the apparently corresponding bands must, however, await the isolation of antibodies specific to the bands. The reason why sample 4 (Figs. 1 and 3), which was found to be negative for thyroglobulin by haemmaglutination at various dilutions [17] should exhibit such a strong reaction with M_r 210,000 is unclear. It is possible that it

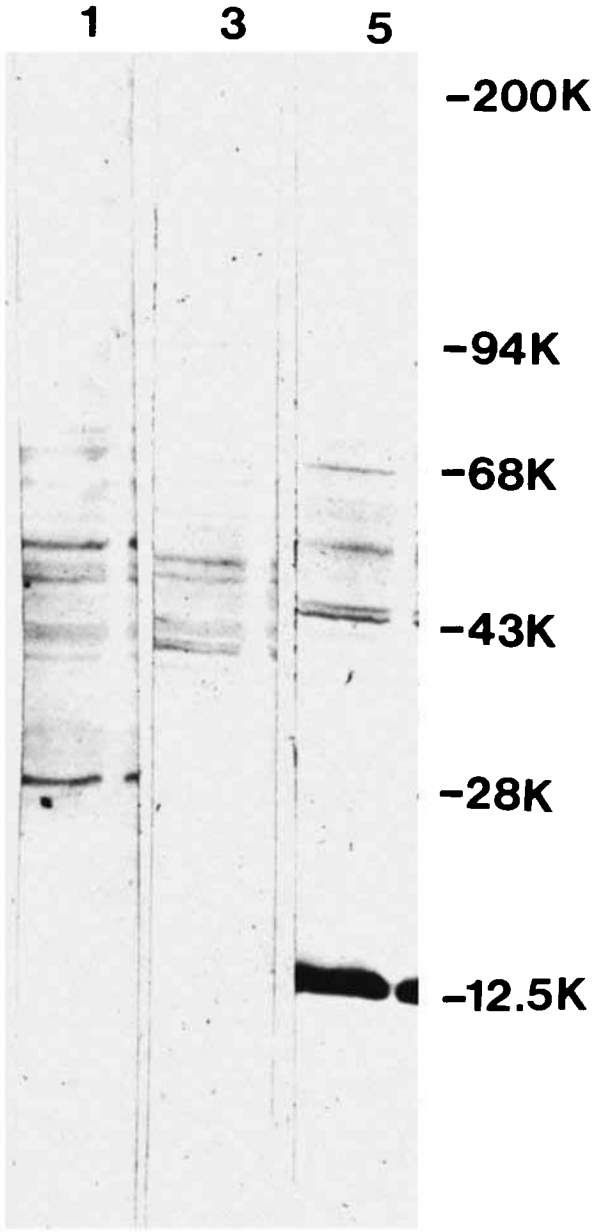

Fig. 3. Pattern of interaction of selected Hashimoto's IgG with plasma membrane protein resolved under reducing conditions and transferred to nitrocellulose paper. The numbers above the lanes correspond to the samples numbered in Figure 1. A faint band may be seen at $M_r \sim 180K$ in all three lanes, suggesting that all these sera have low concentrations of antibodies against pp 210, which migrates faster under reducing condition. The "unique" patterns of antibody interaction described in the text are relative rather than absolute. The 197K band can no longer be visible and a distinct $M_r = 28,500$ band appears in Lane 1. The fine band $M_r = 66,000$ in the three lanes probably represents a TSH receptor subunit [see Islam et al, 1983a].

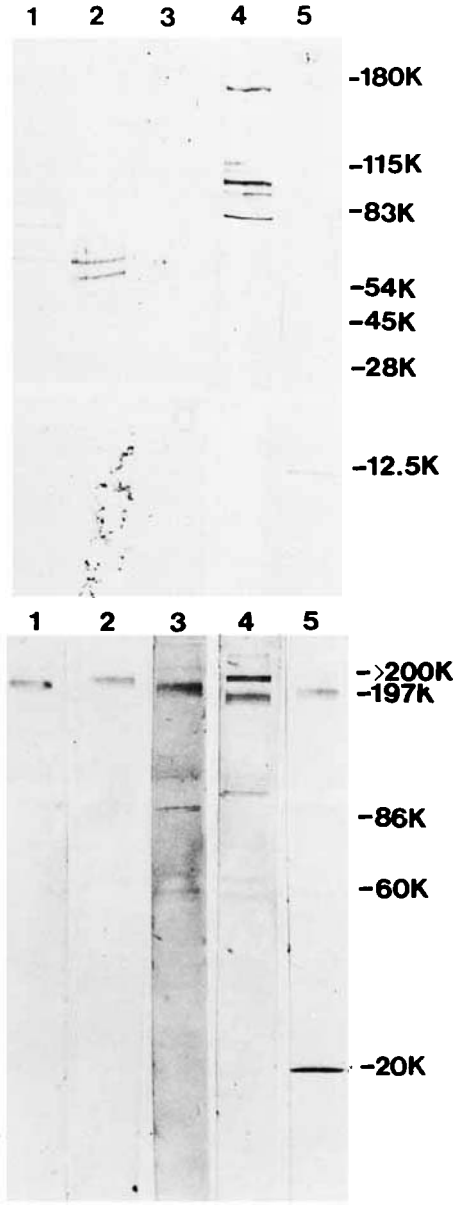

Fig. 4. Binding of light microsome proteins resolved in the presence (upper panel) or absence (lower panel) of reductant. Note should be made of the substantial reduction in the mobility of several bands upon reduction of disulphide bonds within peptide chains. Numerals above the lanes refer to sample numbers. The patterns are generally comparable to those seen for plasma membranes in Figures 1 and 3.

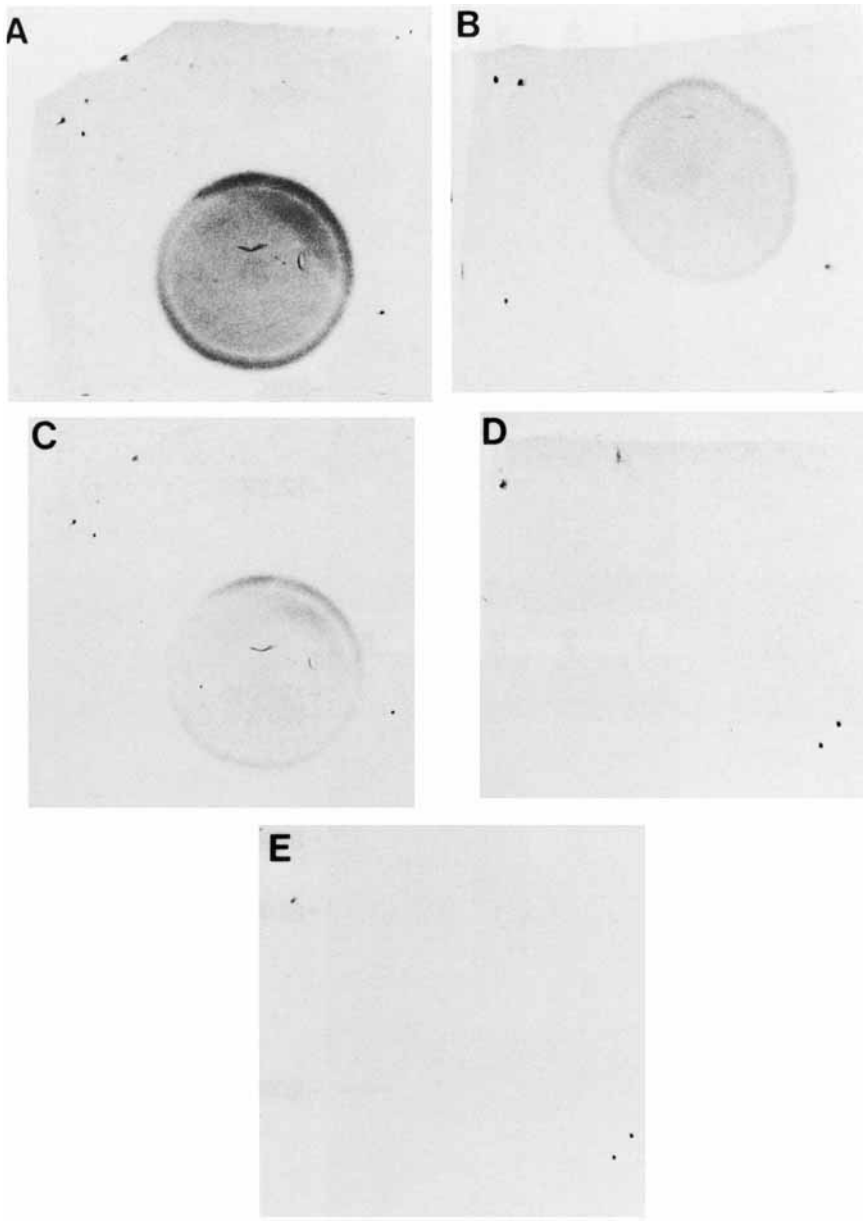

Fig. 5. Interaction of Hashimoto's and Graves' IgG with affinity-purified TSH receptor. The interaction of Hashimoto's IgG (2 mg/ml) (A) with purified receptor was not inhibited by prior exposure to 500 mU/ml bTSH (B) by contrast the binding of Graves' IgG (1 mg/ml) (C) is virtually completely inhibited by preincubation with bTSH (D). Control human IgG is shown (E). The affinity purified TSH receptor is composed ~ 90% of holoreceptor or breakdown products; holoreceptor constitute ~ 70% of the preparation.

Fig. 6. Specific binding of monoclonal antibody TR 17.193 with pp 210. Protein blots of thyroid plasma membranes resolved under nonreducing conditions were allowed to bind to monoclonal IgG and the bound antibody identified by a mouse specific anti-IgG antibody and chromogen.

may interact with determinants on thyroglobulin degradation products, which are masked in the native protein. M_r 210,000, on the other hand, may be a major form of thyroglobulin associated with membranes, which may also account for specificity of monoclonals raised as a result of immunization with plasma membranes. The remaining samples show faint reaction with M_r 210,000 on protein blots of membranes resolved in the absence of reductant. A more distinct and consistent band at M_r 180,000 was seen with both plasma and microsomal membranes resolved under reducing conditions. Strong reactions were, however, obtained with 15 μg of blotted porcine thyroglobulin which emphasizes the sensitivity of the method.

Lastly, we tested the ability of Hashimoto's IgG to influence basal and TSH-driven AC activation. Two of the IgG preparations activated AC, two inhibited TSH-stimulated AC activation, and three were without effect (see Fig. 1).

DISCUSSION

The porcine thyrotropin receptor is a heterotetrameric $M_r \sim 200,000$ glycoprotein [5,9,13]. It is composed of two covalently bonded M_r 35,000 subunits which interact non-covalently with two heavy chains (M_r 66,000). The TSH bindings and the integrity of the receptor depend on reduced -s-s- bridge(s) between the two light chains. Both light and heavy chains are exposed on the epithelial cell surface. We found that Graves' IgG binds to the M_r 200,000 receptor band on protein blots of thyroid membranes; this interaction was blocked by native TSH and cannot be elicited with membranes resolved under reducing conditions before protein blotting [9]. We now show that Hashimoto's IgG binds to the TSH receptor; this binding, however, cannot be blocked by TSH. These findings are in keeping with observations based on whole cells or crude membranes [19,20]. The M_r 197,000 band with which Hashimoto's IgG specimens interact is not related to thyroglobulin because its molecular weight is different from that of thyroglobulin degradation products. The TSH binds to this band specifically and with high affinity. Moreover, this interaction and that

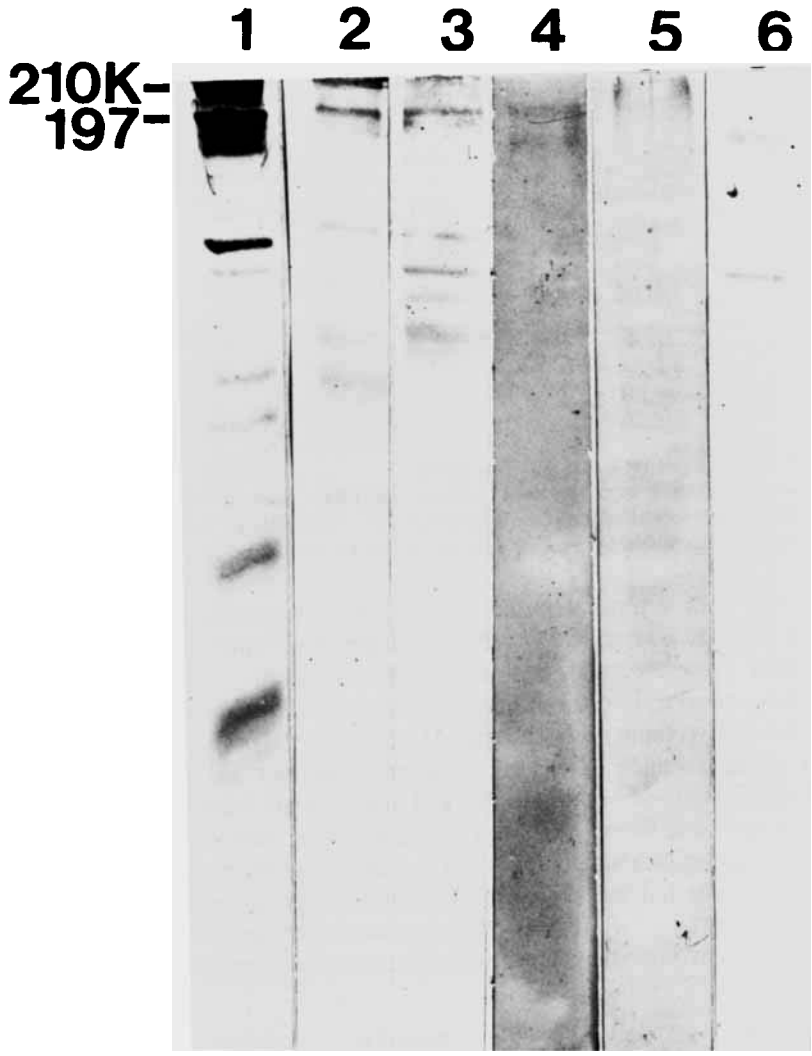

Fig. 7. Interaction of Hashimoto's Graves' IgG, and anti-thyroglobulin antibody with commercial thyroglobulin and plasma membranes resolved under nonreducing conditions. Lanes: 1) Interaction of thyroglobulin with anti-thyroglobulin. 2) Interaction of sucrose gradient purified thyroid plasma membranes with anti-thyroglobulin. 3) Binding of Hashimoto's IgG to protein-blotted thyroglobulin. 4) Binding of Hashimoto's IgG to protein blots of thyroid plasma membranes. 5) Interaction of Graves' IgG with thyroglobulin. 6) Interaction of Graves' IgG with thyroid plasma membrane. The rabbit polyclonal antibody interacts with a variety of thyroglobulin related peptides including ones of M_r 210,000 and 180,000. Hashimoto's IgG, which were negative by hemmagglutination assay are strongly positive to these two polypeptide bands both when allowed to interact with thyroglobulin or plasma membranes. Graves' IgG results in faint bands in thyroglobulin which can be barely seen but a distinct band with the TSH receptor. The band of M_r 165,000 on lane 6 is unrelated to thyroglobulin and is often obtained with normal rabbit IgG.

with Graves' and Hashimoto's IgG disappear when investigated following treatment of the receptor with disulphide bond reductants [9,13]. Lastly, Hashimoto's IgG specimens interact with TSH affinity-purified receptor, which excludes a possible incidence in the molecular weight of the receptor and the microsomal antigen. It thus appears that Graves' and Hashimoto's IgGs bind to dissimilar moieties on the TSH receptor from that to which TSH and Graves' IgG bind. Graves' IgG also may exhibit greater binding affinity than Hashimoto's IgG, in that optimal binding with receptor is found with 1 mg/ml of the former but only with 1.5–2 mg/ml of Hashimoto's IgG [9].

It was recently found that when Graves' disease IgG was absorbed with thyroid plasma membranes antimicrosomal antibodies were co-eluted with the putative anti-TSH receptor antibodies [21]. Only Graves' IgG was, however, obtained when guinea pig adipocyte membrane was the absorbent instead [21], although not all investigators have been as successful in dissociating these two activities [19]. The IgG molecules with thyroid-stimulating activity could be dissociated to some degree from those with antimicrosomal specificity by isoelectric focusing [22].

Hashimoto's IgG interacted with the TSH receptor present on thyroid plasma membranes, as well as on light microsome preparations. Independent immunocytochemical studies from this laboratory suggest an abundance of TSH, Graves' IgG, and Hashimoto's IgG binding sites within thyroid cells, whereas Graves' IgG binding was virtually completely with TSH. Hashimoto's antibody interaction was not affected by this treatment [23].

Our findings of sharing antigens, including the TSH receptor, between "light microsomes" and plasma membrane is consistent with the realization that light microsomes are identical with the exocytotic vesicles which contribute to the plasma membrane of the apical cell surface [24]. Khoury et al [25] also found that the "microsomal" autoantigen was present on both light microsomes and thyroid cell surface. The microsomes do contain free as well as 2 species of microsome-bound thyroglobulins. The latter differ in iodine and sialic acid content from 19S thyroglobulin [26]. These differences may well be of relevance to the fine specificity of anti-thyroglobulin autoantibodies.

Our finding that anti-microsomal antibodies interact with an epitope of the TSH receptor is in keeping with the multiplicity of antibodies apparently directed against this receptor identified in the sera of patients with autoimmune thyroid disease. These include antibodies with thyroid growth [27] or thyrotropin-receptor blocking [28] activities; some of the latter block thyroid growth in utero [29]. The description of these antibodies as well as monoclonal raised against the receptor will help better understand receptor structure and function [30].

ACKNOWLEDGMENTS

We thank Dr. Victor Gopalakrishnan for enzyme measurements of subcellular fractions and Miss Gail O'Brien for the art work and secretarial assistance. Supported by grant MA-7908 from the Medical Research Council of Canada.

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