Journal of Cellular Biochemistry 34:151–162 (1987)

Use of Anti-Idiotypic Antibodies as Probes for the Interaction of TSH Subunits With Its Receptor

Rosario Briones-Urbina, M.N. Islam, J. Ivanyi, and Nadir R. Farid

Thyroid Research Laboratory, Health Sciences Center, St. John's, Newfoundland, Canada, A1B 3V6 (R.B.-U., M.N.I., N.R.F.) and The Wellcome Research Laboratories, Bechenham, Kent, England, BR3 3BS (J.I.)

TSH is a heterodimeric glycoprotein hormone, whose dissociated subunits are without biological activity. This has precluded the assessment of the relative contribution of each subunit to hormone action. We have raised anti-idiotypes to monoclonal antibodies specific, respectively, for the α and β hTSH subunits. The anti-β anti-idiotype inhibited ^{125}I-hTSH binding to the β subunit-specific monoclonal quantitatively, whereas ^{125}I-hTSH binding to the α subunit-specific monoclonal was not inhibited by anti-α anti-idiotypes, suggesting that only the former is an "internal image" anti-idiotype. Neither of the two anti-idiotypes nor equimolar mixtures thereof inhibited ^{125}I-bTSH binding to thyroid membranes, even though radiolabelled anti-idiotypes showed saturable binding to thyroid plasma membrane which was inhibited 41–65% by bTSH. Each anti-idiotype alone caused 9% inhibition (compared to 50% by NRIgG) of thyroid plasma membrane adenylate cyclase. Equimolar mixtures (125 μg/ml IgG of each anti-idiotype) induced enzyme activity equivalent to 85% of that of 250 mU/ml of TSH. The TSH-like action of the two anti-idiotypes was also reflected in their capacity to increase (450% by 250 μg/ml IgG compared to normal rabbit IgG) the uptake of ^{131}I into isolated thyrocytes and to promote the organization of such cells into follicular structures. At 250 μg/ml, anti-β anti-idiotype promoted the organization of small follicles and only at a concentration of 500 μg/ml did it enhance ^{131}I uptake.

Abbreviations used: AC, adenylate cyclase; anti-id, anti-idiotype; α anti-id and β anti-id, antibodies raised against monoclonals specific for the α and β subunits, respectively, of TSH; bTSH, bovine thyrotropin; DEAE, diethylamino ethyl; FCS, fetal calf serum; FSH, follicle stimulating hormone; HAT, hypoxanthine-aminopetrin-thymidine; HBSS, Hank's balanced salt solution; hCG, human chorionic gonadotropin; hTSH, human thyrotropin; LH, luteinizing hormone; MEM, minimum essential medium; MoAb, monoclonal antibody; $NaDSO_4$, sodium dodecyl sulphate; NRIgG, normal rabbit IgG; TRIS-HCl, tris (hydroxymethyl) aminomethane hydrochloride.

J. Ivanyi's present address is MRC Tuberculosis and Related Infections Unit, Hammersmith Hospital, London W12 OHS, UK.

Received June 3, 1985; revised and accepted December 17, 1986.

Anti α idiotype was without effect in both assays. Lastly, mixture of anti-idiotypes bound to the ~ 197,000 Mr band (TSH holoreceptor) on protein blots of thyroid plasma membranes resolved on $NaDSO_4$-polyacrylamide gel electrophoresis under non-reducing conditions. Individual anti-idiotypes were without effect.

The TSH α and β subunits apparently deliver two cooperative signals to the receptor and that specificity is associated with the β subunit, while the α subunit is important in enhancing receptor affinity for the heterodimer and in stablizing TSH-receptor complex.

Key words: anti-idiotypic antibodies, thyrotropin subunits, thyrotropin receptor, monoclonal antibody

Thyrotropin (TSH) is a glycoprotein hormone composed of α and β subunits and which stimulates thyroid hormone synthesis by thyroid follicular cells and possibly their replication. The isolated subunits are biologically inactive; upon recombination, they reacquire biological activity and undergo conformational modifications suggesting that unique structural properties of the intact hormone may be needed for activity [1]. Both subunits may thus come in contact with the receptor, although only one may deliver the hormone-specific signal. The lack of biological activities of the dissociated subunits and knowledge of the tertiary structure of TSH have hampered attempts to relate structure of the hormone to its function.

We have used a novel immunological approach to investigate the contribution of the subunits to the biological activity of TSH. A set of anti-idioptypic antibodies raised against a ligand-specific antibody will be specific for antigen-binding domain (paratope) and as such carries an "internal image" of the ligand [2–5]. On this premise, we have immunized rabbits with TSH subunit-specific monoclonal antibodies. We found that a mixture of both anti-idiotypic antibodies, but neither alone, can simulate TSH action, suggesting two signals delivered by the subunits of TSH to its receptor rather than a single signal requiring their combination.

METHODS

Preparation of Monoclonal and Anti-Idiotypic Antibodies

Anti-thyrotropin subunit-specific monoclonal antibodies were obtained by immunizing Lou rats with 100 μg highly purified hTSH in complete Freund's adjuvant and 5 months later immunizing them intravenously (50 μg in solution). Spleen cells removed 3 days after a second intravenous dose were fused with Y3-Ag1.2.3. rat myeloma cell line [6]. Supernatants of hybrids selected by culture in HAT medium were tested for their ability to precipitate radiolabelled glycoprotein hormones [7]. An IgG monoclonal Gc73 was interacted specifically with hTSH, whereas TS28, of IgM isotype, cross-reacted completely between hTSH, hLH, hFSH, and hCG, ie, were specific for human α subunit.

Two New Zealand rabbits each were immunized with 100 μg Gc73 or TS28 monoclonal antibodies in Freund's complete adjuvant. At the end of 1 month the rabbits were boosted every 2 weeks with 50 μg antigen in incomplete adjuvant five times; 2 weeks after the last injection, the animals were exsanguinated. Immunoglobulin G from immunized as well as control rabbits were obtained by DEAE cellulose chromatography [8] and passed through a rat Ig-Reactigel (Bio-Ril, Montreal, P.Q.) affinity column to remove isotype- and allotype-specific antibodies.

Binding of Monoclonals to Anti-Idiotypes and TSH

Ten micrograms of highly purified bTSH (30 IU/mg) (kindly provided by Dr. John Pierce, UCLA) and 25 μg of Gc73, TS28, normal rabbit, anti-idiotype IgG or normal rat Ig, were iodinated [9]. Labelled proteins were separated on Ultrogel AcA54 columns (Reactifs IBF, France). ^{125}I-hTSH (specific activity 50 mCi/mg) was purchased from Nuclear Medical Laboratories (Irving, TX).

Monoclonal antibodies TS28 or Gc73 (100 μg/ml each) were preincubated for 14 h at 4°C in the absence or presence of 50–200 mU/ml of bTSH (USV Laboratory, Mississauga, Ontario) or 125–500 μg/ml of specific anti-idiotypes. Radiolabelled bTSH or hTSH (~ 20,000 cpm) was added to the samples and incubated for a further 30 min at 37°C. In another series of experiments, ^{125}I monoclonals and normal rat immunoglobulins (~ 50,000 cpm, ≈ 15 ng) were incubated with specific anti-idiotypic antibodies or NRIgG (100–1,000 mg/ml) for 60 min at 37°C. Bound from free radiolabelled ligand was then separated by centrifugation at 32,000g for 5 min in the presence of 12% polyethylene glycol 6,000 in bovine gammaglobulin and 1 M NaCl [10].

Interaction of Anti-Idiotypes With Thyroid Plasma Membranes

Porcine thyroid plasma membranes (100 μg) [5] suspended in 50 μl of binding buffer (25 mM NaCl, 20 mM TRIS-HCl, pH 7.4) were incubated for 30 min at 37°C with 0.5 pmol of ^{125}I-bTSH, with or without different concentrations of native bTSH or 100 μg of rabbit IgG (NRIgG, individual anti-idiotypic IgGs, or equal mixtures of anti-idiotypic IgGs) in a final assay volume of 200 μl. Binding of ^{125}I-labelled anti-idiotypic antibodies or NRIgG (~ 10 ng, 4×10^4 cpm) to thyroid plasma membranes was also tested in the presence or absence of 500–1,000 μg/ml of native IgG or 50–1,000 mU/ml of bTSH to determine, respectively, the saturability of their binding sites and their relationship to those for TSH. The tubes were centrifuged at 32,500g for 20 min and the sediment counted.

Interaction of Protein Blots of Thyroid Plasma Membrane With Anti-Idiotypes

Thyroid plasma membranes (100 μg) were resolved on a 7.5–15.0% linear gradient polyacrylamide slab gel [11], in the presence or absence of 5% β-mercapto-ethanol and electrotransferred onto nitrocellulose paper [12] overnight at 200 mA. Individual lanes were cut and processed either for binding to bTSH or to anti-idiotype IgG, as previously described [12]. For TSH binding, the nitrocellulose paper strips were washed with 20 mM TRIS-HCl, pH 7.4, whereas strips studied for antibody binding were incubated with anti-idiotype IgG for 14 h at 4°C and then washed several times with 40 mM TRIS-HCl, 200 mM NaCl, pH 7.4. Specificity of interaction of antibodies with blotted bands was determined by preincubation of the paper strips with 500 mU/ml of TSH, hCG, or porcine insulin. To determine molecular weights of transferred, standard protein markers (Sigma, St. Louis, MO) were included.

Influence of Monoclonals and Anti-Idiotypes on Thyroid Adenylate Cyclase Activity

The effect of monoclonals Gc73 and TS28, anti-idiotypes, and normal rat Ig or NRIgG on adenylate cyclase activity of thyroid plasma membranes was tested with

some modifications of a previously described method [5,13]. Forty micrograms, rather than 20 μg [5], of thyroid plasma membranes were pre-incubated for 10 min with either 200 mU/ml bTSH, or 250 μg/ml monoclonals, anti-id IgG, normal rat Ig, or NRIgG, respectively. Where the effect of monoclonals on TSH-driven AC was investigated, these antibodies (or normal rat Ig as control) were pre-incubated for 10 min before or simultaneously with bTSH. cAMP was measured using a protein-binding assay [14]. Saturating doses for cAMP were found to be 200–250 mU/ml bTSH.

Radioiodine Uptake and Follicular Organization of Dispersed Thyroid Cells

Single cell suspensions of porcine thyroid cells were prepared as previously described [15], with minor modifications.

Cells (10×10^6) in 5 ml MEM/FCS were then allowed to plate for 24 h in 6-well tissue culture plates (Costar, Cambridge, MA). Plated cells were incubated for 12 h with 100 mU/ml bTSH, anti-idiotype IgG, or NRIgG (250 μg/ml) [5], scraped, and adjusted to a concentration of 1×10^6 cells/well. ^{131}I-Na (1×10^5 cpm) (New England Nuclear, Boston, MA) were added per well and incubated for 30 min at 37°C. Cells were then chilled at 4°C, placed in Eppendorff microassay tubes, washed twice, and the radioiodine content of the pellet determined.

Follicular organization of dispersed thyroid cells by bTSH (100–200 mU/ml), NRIgG, and anti-id IgG (250–500 μg/ml) were studied [5]. Medium was changed at days 4 and 7 of culture and induction of follicles examined under a light microscope [5].

Where applicable, statistical comparisons were made by the Mann-Whitney U test [16].

RESULTS AND DISCUSSION

Monoclonals Gc73 and TS28 exhibited complete specificity for human α and β subunits, respectively. Both MoAbs bound less than 2.0% ^{125}I-bTSH added, not different than background. TS28 interaction with ^{125}I-hTSH was inhibited by hCG, hLH, and hFSH, suggesting that this monoclonal is specific for the human α subunit common to these hormones [1]. Gc73, by contrast, is specific for hTSH and has, therefore, been designated β-subunit-specific. The dissociation constants (K_d) of the MoAbs were 3.7×10^{-9} M for Gc73 and 5.5×10^{-9} M for TS28.

Only Gc73 inhibited binding of ^{125}I-bTSH to thyroid plasma membranes, 500 μg/ml of monoclonal IgG resulting in 52% inhibition (Table I). Neither MoAb influenced thyroid membrane basal AC activity. Allowed to interact with membranes prior to the addition of bTSH, both MoAbs at concentrations of 250 μg/ml inhibited bTSH-driven cyclase activation by 58–75% (Table II). When MoAbs (250 μg/ml) were added, instead, to the membrane simultaneously with hormone, the bTSH-associated cyclase activation was enhanced 69% by TS28 and 59% by Gc73, compared to that achieved by bTSH alone.

Whereas MoAb Gc73 and TS28 display exquisite specificity for the α and β subunits of hTSH, the TSH receptor displays homology across species [17]. We, therefore, entertained the possibility that the MoAb may bind to the receptor. This was excluded because 3.5% or less of ^{125}I-MoAbs counts added were bound to thyroid membranes. Binding of bTSH to specific sites on plasma membranes is

TABLE I. Inhibition of ^{125}I-bTSH Binding by the TSH Subunit-Specific Monoclonals

Reagents added	Percentage binding to membranes (± SD)
None	14.0 ± 1.6[a]
Monoclonal TS28	
250 μg	12.2 ± 2.0
500 μg	12.8 ± 1.8
Monoclonal Gc73	
250 μg	8.8 ± 1.5*
500 μg	6.8 ± 1.4*
Normal rat Ig	
500 μg	12.9 ± 2.1

[a]Total counts added ~ 20,000 cpm. Nonspecific counts (in presence of 1,000 mU/ml) were 3.9 ± 0.6%.
*$p < 0.05$, compared to controls.

TABLE II. Effect of bTSH Subunit-Specific Monoclonals (MoAb) on TSH-Stimulated Adenylate Cyclase Activity*

	MoAb added after bTSH		MoAb added with bTSH	
Assay conditions	cAMP (pmol/mg/min ± SD)	Change (%)[a]	cAMP (pmol/mg/min ± SD)	Change (%)[a]
Basal	134 (± 44)	—	142 (± 38)	—
TSH[b]	224 (± 57)	+ 67.0	225 (± 53)	+ 59
MoAb TS28[c]	148 (± 30)	+ 10.0	175 (± 49)	+ 23
TSH + MoAb TS28	147 (± 30)	+ 9.7	325 (± 70)	+ 128
MoAb Gc73	145 (± 14)	+ 8.0	190 (± 29)	+ 15
TSH + MoAb Gc73	121 (± 8.9)	− 9.7	310 (±77)	+ 118
Normal rat IgG	155 (± 9.0)	+ 15.6	ND[d]	—
TSH + normal rat IgG	200 (± 18)	+ 49.0	ND	—

*By the Mann-Whitney U test, MoAb TS28 and MoAb Gc73 show significant ($p < 0.05$) inhibition of TSH-dependent cAMP generation (left panel) and stimulation (right panel) compared to TSH alone.
[a]Percentage change with reference to cAMP level; + = stimulation, − = inhibition. SD are not included, for convenience.
[b,c]Throughout, bTSH at a concentration of 200 mU/ml and MoAb and rat IgG at 250 μg/ml were used.
[d]Not done.

associated with conformational changes in the α/β dimer [18] such that sites with which the MoAbs can interact become available. MoAb binding to the respective TSH subunit enhances adenylate cyclase activation, probably through receptor clustering. Prior incubation of the MoAbs may interfere with the conformatonal changes in TSH subunits necessary for receptor signalling. That MoAb Gc73 can inhibit ^{125}I-bTSH binding to thyroid membranes (Table I) suggests that the epitope exposed by hormone binding to the receptor is important for recognition, while the epitopes for both MoAb may be relevant to the effector function of TSH heterodimer. We cannot exclude the possibility that MoAbs bind to receptor epitopes exposed after interaction of bTSH with the receptor.

The anti-id preparations represent breakthrough fractions of immune rabbit IgG cycled twice through rat immunoglobulin affinity columns. The amount of IgG molecules in these preparations estimated to be anti-idiotype in nature was 5–7%.

Immunoglobulins obtained from rabbits immunized with either TS28 or Gc73 were tested in all possible combinations. Only the combination of IgGs (200 μg/ml each) from rabbit 1 immunized with TS28 (α anti-id) and rabbit 3 immunized with Gc73 (β anti-id) exhibited biological activity.

The anti-id preparation precipitated the relevant radiolabelled MoAb, α anti-id precipitated proportionately less MoAb than β anti-id (Table III). Specific binding amounted to 11% in the case of anti-id to MoAb TS28 and 35% for the anti-id to MoAb Gc73. We were unable to demonstrate inhibition of binding of ^{125}I-hTSH to TS28 by the corresponding anti-id up to a concentration of 500 μg/ml. On the other hand, 48.2% of the label was displaced from Gc73 by the same concentration of specific anti-id (Table IV). Thus, of the two anti-ids found to be useful, only the anti-Gc73 antibody represents an internal image anti-idiotype.

The anti-α anti-id, while unable to influence ^{125}I-hTSH binding to TS28, was capable of binding to sites on thyroid plasma membranes interactive with TSH and demonstrated cooperative interaction [19] with anti-β anti-id (see below). This interaction between the two anti-ids chosen for study was specific; it was not demonstrable with normal rabbit IgG nor IgG from rabbits immunized with TS28 but without

TABLE III. Interaction of Monoclonals With Their Anti-Idiotypes

	Counts per min	Percentage binding (± SD) of radiolabelled MoAb
^{125}I-TS28 (TC)[a]	45,000	—
+ α anti-id (500 μg/ml)	14,219 (± 299)	31.6 (± 2.1)
+ normal rabbit IgG (500 μg/ml)	8,110 (± 414)	18.02 (± 5.1)
^{125}I-Gc73 (TC)[a]	54,000	
+ β anti-id (500 μg/ml)	31,646 (± 5,060)	58.6 (± 16)
+ normal rabbit IgG (500 μg/ml)	4,209 (± 130)	7.79 (± 3.1)
^{125}I-Normal rat Igs (TC)[a,b]	49,125	
+ α anti-id (500 μg/ml	10,086 (± 349)	20.5 (± 3.46)
t+ β anti-id (500 μg/ml)	7,087 (± 352)	14.4 (± 4.7)

[a]Total counts.

[b]These Igs were obtained by ammonium sulphate precipitation including both IgG and IgM determined by immunodiffusion. The ideal controls would have been MoAb IgM (for TS28) and MoAb IgG (for Gc73) antibodies specific for antigens other than TSH; such antibodies could not be obtained.

TABLE IV. Inhibition of Monoclonal Antibody Binding of ^{125}I-hTSH by the Anti-Idiotypes

	Percentage binding (± SD)	Percentage change
^{125}I-hTSH + TS28 (100 μg/ml)	64.94 (± 3.07)	—
+ α anti-id (125 μg/ml)	70.83 (± 0.88)	+ 9.1
+ α anti-id (250 μg/ml)	70.2 (± 1.08)	+ 8.1
+ α anti-id (500 μg/ml)	71.0 (± 3.25)	+ 9.3
^{125}I-hTSH + GC73 (100 μg/ml)	65.58 (± 3.63)	—
+ β anti-id (125 μg/ml)	59.1 (± 1.47)	− 5.56
+ β anti-id (250 μg/ml)	54.0 (± 1.57)	− 13.71
+ β anti-id (500 μg/ml)	32.4 (± 0.36)	− 48.23

biological activity. The anti-α anti-id are probably complementary to framework determinants in close proximity to the antigen binding site [20; C. Bona, personal communication].

Neither class of anti-id antibodies nor combinations thereof resulted in the inhibition of ^{125}I-bTSH binding to thyroid plasma membrane above that caused by pre-immune rabbit IgG.

Three percent of radiolabelled α anti-id IgG and 2.5% of β anti-id IgG bound to thyroid plasma membranes (Fig. 1). Forty percent of ^{125}I-α anti-id and 65% of radiolabelled α/β anti-id were inhibited by an excess of the corresponding unlabelled antibody (Fig. 2). The binding of two radiolabelled anti-ids appeared to be additive and was inhibited by 500 mU/ml of native TSH. Binding affinities of $\sim 2.0 \times 10^{-7}$, $\sim 1.0 \times 10^{-7}$, and $\sim 5 \times 10^{-8}$ were calculated for anti-α, anti-β, and anti-α binding to the TSH receptor.

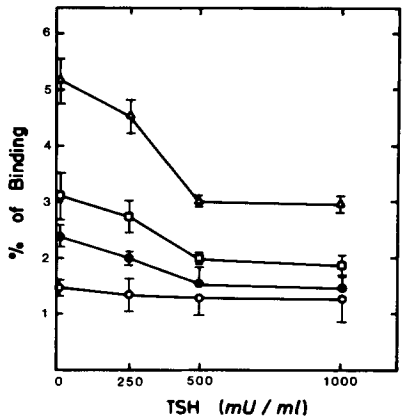

Fig. 1. The binding of ^{125}I-anti-idiotypic antibodies to thyroid plasma membranes and inhibition by TSH. Binding of anti-α + anti-β anti-ids is additive and displacable by TSH. Bars represent $\pm$ SD. Binding of radiolabelled individual anti-ids ($p < 0.05$) and their combination ($p < 0.01$) was significantly greater than NRIgG. ○, NRIgG; ●, anti-β anti-id; □, anti-α anti-id; △, anti-α + anti-β anti-id.

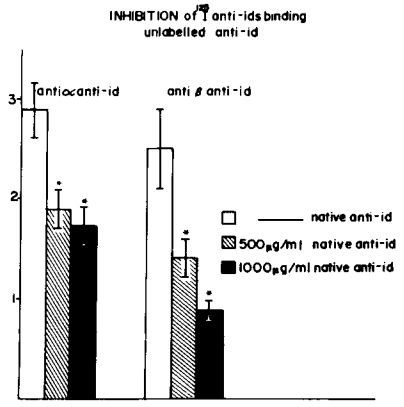

Fig. 2. Effect of unlabelled anti-idiotypic antibodies on the binding of ^{125}I-anti-idiotypic antibodies to thyroid plasma membranes. Binding displacement by native anti-ids is dose-dependent. Bars represent $\pm$ SD; *, $p < 0.05$.

By contrast to the additivity of ^{125}I-anti-id binding to thyroid membranes, marked enhancement was found of the effect of α anti-idiotypic and β anti-idiotypic antibodies on AC. α Anti-id and β anti-id increased basal cyclase activity above the level seen with an equal amount of NRIgG. An equimolar mixture of the two antibodies increased cyclase activity to 85% of that achieved with 250 mU/ml of bTSH. (Fig. 3). The more than additive influence of the two anti-idiotypes suggests cooperative interaction [19]. This could entail the binding of one antibody to thyroid membrane, increasing binding affinity for the second without change in number of binding sites, the binding of both antibodies increasing the agonistic effect of one antibody or allosterically changing the efficiency of receptor/effector coupling. These considerations could adequately explain why the specific binding of ^{125}I-anti-idiotypes is additive, whereas their effect is cooperative.

Both uptake of ^{131}I by thyroid cells and their organization into follicular structures are induced by TSH and are mediated, at least in part, by cAMP [5]. α Anti-id was without effect on the uptake of ^{131}I compared to NRIgG. β Anti-id stimulated ^{131}I uptake at the higher concentration, while the mixture of α anti-id and β anti-id increased the percentage of incorporation to a level comparable to that obtained with 100 mU/ml of TSH (Fig. 4).

NRIgG and α anti-id had no effect and β anti-id promoted some degree of follicle organization. The mixture of α anti-id and β anti-id, on the other hand, resulted in well-formed follicles in which epithelial cells were actively engaged in thyroglobulin synthesis (Fig. 5).

The apparent cooperative effect of the two anti-id preparations was also noted when we attempted to visualize protein-blotted TSH receptor. When allowd to interact with protein blots of thyroid plasma membranes resolved by $NaDodSO_4$ polyacrylamide gel electrophoresis, neither α anti-id or β anti-id resulted in discernible signals at the 197,000 Mr level. A mixture of the two antibodies, however, gave a reproducibly prominent band (Fig. 6), corresponding to the band (M_r ~ 197,000) to which bTSH (lane 1) was bound [12,21]. Binding of the anti-id mixture to ~ 197,000 Mr

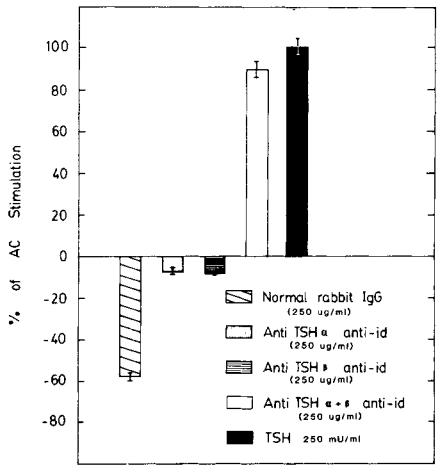

Fig. 3. Stimulation of adenylate cyclase activity by anti-idiotypic antibodies. The effect of combined anti-α anti-β anti-idiotypic antibodies is more than additive and comparable to the effect of 200 mU/ml of TSH.

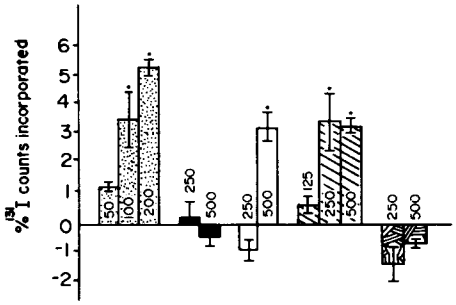

Fig. 4. The effect of anti-idiotypic antibodies on iodine uptake by thyroid cells. bTSH shows a dose-dependent increase in ^{131}I uptake by the cells, NRIgG and corresponding amounts of anti-α anti-idiotype inhibition. Anti-β anti-idiotype results in increased ^{131}I uptake at a dose of 500 μg/ml. By contrast, the same capacity of ^{131}I uptake is achieved with 250 μg each of anti-α + anti-β. Bars represent $\pm$ SD: *, $p < 0.05$. ▨, bTSH; ■, α anti-id; □, β anti-id; ▧, α anti-id + β anti-id; ▨, NRIgG.

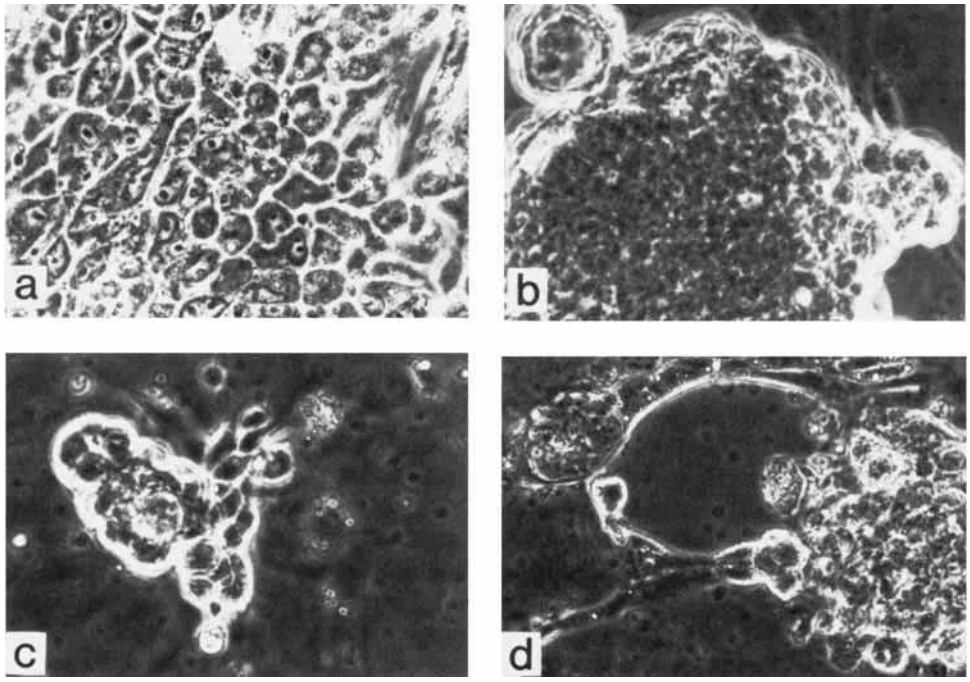

Fig. 5. The influence of anti-idiotypes on the organization of thyrocytes to follicles. Photographs were taken on day 7 of culture with a phase contrast objective (25×–2×) through a green filter. **a)** Anti-α anti-idiotype is associated with elongated cells growing in a monolayer. **b)** TSH 100 mU/ml. A large follicle is shown. **c)** Anti-β results in a "half-melon" appearance of follicles, engaged in thyroglobulin synthesis. **d)** Anti-β + anti-α anti-ids observe the follicular type structure of the cultured cells. Normal rabbit IgG results in a monolayer similar to that by normal IgG (not shown).

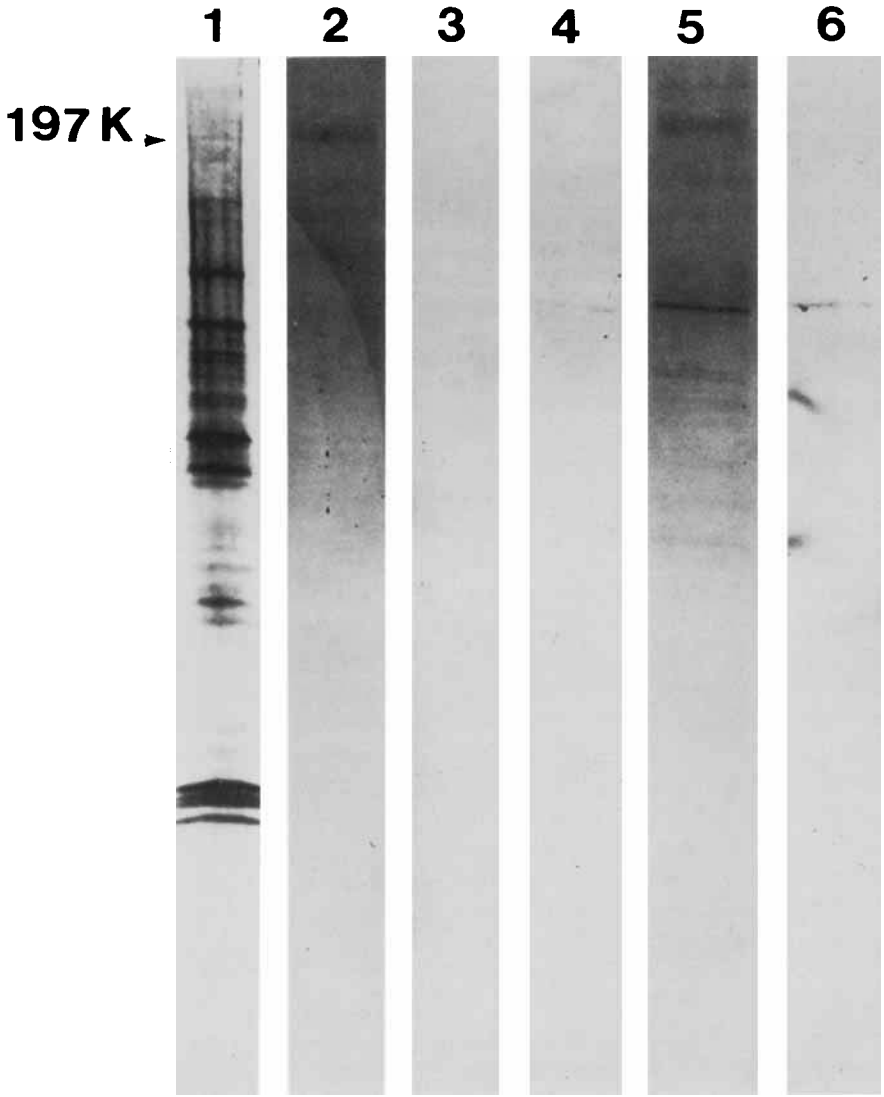

Fig. 6. Interaction of protein blots of thyroid plasma membrane polypeptide resolved by SDS gel electrophoresis with b-TSH anti-idiotypes and normal rabbit IgG. **Lane 1**: Bands transferred from non-reducing $NaDSO_4$ gels and stained with Coomassie blue. **Lane 2**: b-TSH (1 U/ml). **Lane 3**: Anti-α anti-id (1 mg/ml). **Lane 4**: Anti-β anti-id (1 mg/ml). **Lane 5**: Anti-α + anti-β anti-ids (1 mg/ml). **Lane 6**: Normal rabbit IgG (1 mg/ml). A positive band is seen at Mr ~ 197,000 with both TSH (lane 2) and the combination of anti-α + anti-β anti-ids (lane 5). Lanes 3, 4, and 6 are negative. Several lower molecular weight bands are seen in some of the lanes. Some are related to normal rabbit IgG, whereas others are specific to the combination of the two anti-idiotypic antibodies. The resolution of the blots shown here does not allow for a confident identification of those bands.

bands was blocked by pre-incubation with TSH but not by hCG or insulin (0.5 U) (not shown).

The apparent paradox between the ability of individual radiolabelled anti-ids to bind to thyroid plasma membranes (Fig. 2) and our inability to demonstrate binding in a sensitive enzyme-linked immunoabsorbent detection system can be resolved by noting that protein blots are extensively washed in 100 mM NaCl to minimize non-specific binding. This procedure removes antibodies of low binding affinity and again emphasizes the cooperative interaction (in increasing each other's binding affinity) of the two anti-idiotypes in binding to the TSH receptor. The presence of multiple epitopes on TSH receptor has been invoked to explain the ability of natural polyclonal and experimental monoclonal antibodies raised to the receptor to stimulate adenylate cyclase without necessarily influencing ^{125}I-TSH binding and vice versa [22]. More stringent structural constraints probably govern antibodies inhibiting TSH binding compared to those which stimulate adenylate cyclase.

The anti-Gc73 and anti-TS28 and anti-idiotypes act as surrogates of α and β TSH subunits, respectively, although only the latter represents an internal image anti-idiotype. The inhibition of binding to thyroid membranes of individual ^{125}I-anti-id by bTSH, the ability of an anti-id cocktail to stimulate adenylate cyclase and events dependent on this step, as well as the specific anti-id binding to a protein blot of a peptide thought to be the TSH receptor [12,21] support the notion that the anti-idiotype interacts with the TSH receptor.

The use of two anti-idiotypes to probe a receptor is noteworthy. Binding of TSH to its receptor induces microaggregation, which is apparently necessary for triggering AC activation [23]. While it is conceivable that the combination of the two anti-ids act via promotion of receptor aggregation, we have to invoke, in addition, the specific binding of each anti-idiotype to separate domains of the receptor and their bivalency in order to account for the slight biologic influence of anti-β anti-id and the cooperative influence of the two anti-ids particularly on the protein-blotted receptor. The apparent lack of biological effect of anti-α anti-id alone may be related to its non-internal image nature or the function of the TSH α subunit in facilitating β subunit action. Given that anti-id are analogous to the respective TSH subunit, it may be surmised that the TSH α and β subunits deliver two cooperative signals to the TSH receptor which are crucial to the conformational changes in the receptor necessary for the stimulation of adenylate cyclase and that signal specificity is associated with the β subunit while the α subunit enhances the effectiveness at the receptor of the subsequent β subunit interaction. Comparison of the interaction of free and receptor-bound bTSH with monoclonal idiotypes also suggests that after binding to the receptor, the bTSH molecule undergoes conformational changes which expose new antigenic sites recognized by the monoclonals. Although we are unable to determine the sequence of TSH subunit binding to the receptor, our interpretation of the data is in general agreement with studies on the interaction of hCG (which shares a common α subunit with hTSH) with its receptor [24]. Apparently, hCG binding is initiated by a specific low affinity interaction of β subunit with the receptor which triggers high affinity α subunit binding with subsequent receptor stabilization [24].

This report emphasizes the potential use of anti-idiotypic antibodies as reagents for probing hormone receptor structure and function, for receptor purification [25–27], as well as better understanding of the idiotype/anti-idiotype network in autoimmune disorders.

ACKNOWLEDGEMENTS

We wish to thank Miss Gail O'Brien for expert secretarial assistance, Ms. Beverley S. Hawe for technical assistance, and Prof. A.D. Strosberg for helpful discussions. This work was supported by MRC grant MA-7908. Presented in part at the UCLA Symposium on Monoclonal Antibodies and Cancer Therapy, Park City, Utah, Jan. 26 to Feb. 2, 1985.

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NOTE ADDED IN PROOF

After completion of these studies, Holder et al (Endocrinology 120:567–573, 1987), reported the monoclonal Gc73 enhanced the effect of TSH in thyroid follicular growth, thyroxine synthesis. The mechinism of Gc73 enhancement of TSH effect was unknown.