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Biologic Activity of Anti-Thyrotropin Anti-Idiotypic Antibody

Nadir R. Farid, Rosario Brioñes-Urbina, and M. Nazrul-Islam

Thyroid Research Laboratory, Health Sciences Centre, St. John's, Newfoundland, Canada A1B 3V6

We raised an antihuman thyrotropin anti-idiotypic antibody and showed that it was active at the thyrotropin receptor. Thus this antibody inhibited ^{125}I b-TSH binding to thyroid plasma membranes, stimulated adenylate cyclase activity through a guanyl nucleotide-dependent mechanism, increased radioiodide entry rate into isolated porcine thyroid follicular cells, and induced such cultured cells to organize into follicles. All these parameters are typical of thyrotropin action. This work raises the possibility that thyroid stimulating antibodies that cause the hyperthyroidism of Graves disease may be, at least in some patients, anti-thyrotropin anti-idiotypic antibodies. It also offers a novel method whereby antireceptor antibodies used in the isolation and characterization of the receptor may be raised from ligands.

Key words: anti-idiotypic antibody, thyrotropin, receptor, thyroid stimulating antibody, Graves disease

The antigen combining site of a specific antibody (idiotype) can itself act as an antigen from which an antibody (anti-idiotype) may arise. That such idiotypes and anti-idiotypes arise in the course of an immune response is firmly established [1,2]; the reciprocal rise of the antibodies [3] and the capacity of anti-idiotypes to induce immunological help or suppression suggests the involvement of anti-idiotypes in the amplification or attentuation, respectively, of the immune response [2]. Seeing that an idiotype comprises a mirror image of an antigenic domain, an anti-idiotype should bear spatial similarities to the antigen. In this case, an anti-idiotype to a ligand (eg, hormone or neurotransmittor) should exhibit biological activity at the corresponding receptor (Fig. 1).

We have produced an antihuman thyrotropin anti-idiotypic antibody and showed that it interacts with the thyroidal thyrotropin (TSH) receptor [4]. That a stimulatory antibody may be raised in this manner is of particular interest because the hyperthyroidism of Graves disease is associated with a thyroid stimulating antibody [5]. The antigen for this antibody has not been definitively identified but is thought to be the TSH receptor itself or plasma membrane moities adjacent to it [5].

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MATERIALS AND METHODS

Raising Anti-Idiotype Antibody

The anti-idiotypic antibody was obtained as described previously [4]. Briefly, Sprague–Dawley male rats were immunized with 50 μg of purified human thyrotropin (Union Chimique Belique, Brussels, Belgium) in complete Freund's adjuvant, boosted 3 weeks later with 20 μg of hormone, and bled 3 weeks thereafter. Immune rat IgG obtained by DEAE-Cellulose (Whatman Ltd., UK) chromatography was applied to an Affigel-b TSH affinity column and the TSH-specific antibody eluted with 0.25 M acetic acid (pH 3.2). Fifty μg of the idiotype in Freund complete adjuvant were used to immunize New Zealand white male rabbits that were boosted 3 weeks later with 30 μg of immunogen and bled 2 weeks after boosting. IgG from the second antibody was extensively absorbed with glutaraledhyde-immobilized, pooled Sprague–Dawley IgG to remove isotypic and allotypic specificities [6].

Radioreceptor and Adenylate Cyclase Assays

Crude and highly purified thyroid plasma membranes were prepared by standard methods described elsewhere in detail [4]. Crude human or porcine thyroid membranes were used to investigate the capacity of anti-id to inhibit ^{125}I b TSH binding and porcine membrane was used only to examine the characteristics of binding of radiolabelled anti-id. One hundred μg of porcine membranes bound an average of 9.4% of 0.5 pM of ^{125}I-b TSH; binding was inhibited to half-maximal by 1.0 mU/ml of native b TSH.

Purified plasma porcine membranes were used to investigate the effects of anti-id, b TSH, and normal rabbit IgG (NR IgG) upon adenylate cyclase activation [4] in the presence or absence of 10 μM p, guanyly1-5'-imidodiphosphate (Gpp(NH)p). Cyclic AMP generated [7] was measured by the protein-binding method [8].

Radiiodination of b TSH, anti-id IgG, and NR IgG were carried out by the lactoperoxidase method [9].

Iodide Transport and Follicular Organization by Dispersed Thyrocytes

Porcine thyroid cells were obtained by mild collagenase digestion of thin thyroid slices followed by centrifugation over 8% bovine serum albumin [4].

A 2.5 × 10^6 thyrocyte suspension in 12 × 75-mm plastic tubes (Falcon Plastic Co., Cockeysville, MD) were incubated with b TSH, anti-id, or NR IgG for different intervals up to 4 hr in a shaking water bath at 37°C in a 95% O_2/5% CO_2 atmosphere. At the end of these intervals, 10^5 cpm of $Na^{131}I$ (New England Nuclear, Boston, MA) were added per tube and the incubation continued for 10 min at room

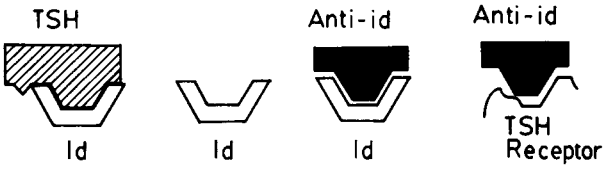

Fig. 1. The rationale for raising anti-TSH anti-idiotypic antibodies. The first antibody (id) is shown as fitting in a complementary manner with the receptor binding determinant of TSH. The second antibody (anti-id) fits the first in the same way and is in fact homologous to TSH. It would be anticipated that anti-id will be reactive with the same determinants on the TSH receptor as TSH.

temperature and the supernatants saved. After washing the cell pellet twice with Krebs Ringer buffer (KRB), the pellet and supernatant were counted and percentage of radiiodine uptake by the thyroid cells calculated [4].

To investigate the capacity of anti-id to induce follicular organization of thyroid cells, 8×10^5 of these cells suspended in McMoy's 5a modified medium (Grand Island Biological Company, Grand Island, NY) supplemented with 10% fetal calf serum and 1% antibiotics were cultured in Lab-tek slides (Lab-tek Division, Miles Labs, Naperville, IL) in the presence of different concentrations of b TSH, NR IgG, and anti-id IgG. The culture medium was changed once on the 4th day. On the seventh day the culture chambers were removed, the slides were washed in phosphate buffered saline (pH 7.2), and examined microscopically.

RESULTS

Two out of three rabbit sera raised against TSH-specific immune rat IgG were found to inhibit ^{125}I-b TSH binding to thyroid plasma membranes. This study reports on the properties of one of these sera after extensive absorption against pooled rat IgG. The antibody did not bind to ^{125}I-b TSH over a wide concentration range (eg, 200 μg precipitated 11% of 20,000 cpm of ligand compared to 8% for background), excluding the possibility that we may have eluted b TSH from the affinity column along with specifically absorbed rat IgG, and that the biological activity of the rabbit antibody was due to antibodies against eluted TSH or complexes thereof.

The rabbit antibody inhibited ^{125}I-b TSH binding to the immunizing specific rat IgG (200 μg causing 85% inhibition) but not by the rabbit preimmune IgG. The first antibody cross-reacted minimally (15% binding at 500 μg IgG) with radio-labeled human chorionic gonadotropin and not at all with insulin or thyroxine. The fact that the rabbit IgG reacts preferentially to an antigen binding site on rat immune IgG specific for TSH proves that the rabbit antibody is an anti-idiotype (anti-id).

The characteristics of ^{125}I-b TSH binding to procine thyroid membranes were as follows: 12.8% $\pm$ 1.4 (SD) of 0.4–0.5 pM ^{125}I-b TSH were bound to 100 μg of membranes with 3.4 ($\pm$ 0.2) of the counts bound representing nonspecific binding. The amount of native b TSH reducing binding of ^{125}I-b TSH to half its maximal value averaged 1.0 mU/ml. Anti-id antibodies caused a dose-dependent inhibition of ^{125}I-b TSH binding to thyroid membranes (Fig. 2).

An average of 3.9% of ^{125}I anti-id bound to crude thyroid membranes. Normal rabbit IgG caused a dose-dependent displacement of bound ^{125}I anti-id to 18% at 200 μg/ml. Native anti-id, by contrast, resulted in a 70% inhibition of binding of ^{125}I anti-id at 200 μg/ml, suggesting that this binding to thyroid membranes is saturable. Binding of ^{125}I anti-id to thyroidal membranes was also inhibited by native b TSH: 160 mU/ml inhibited radiolabeled antibody binding by 64%.

The high endogenous AC activity of porcine thyroid and its limited stimulation by b TSH compared to bovine thyroid was reported previously [10]. In these experiments we found that thyroid plasma membrane AC was insensitive to b TSH treatment [11] in the absence of the nonhydrolazable analogue of GTP, guanylyl imidodiphosphate (Gpp(NH)P) [12]. We carried out a number of experiments to characterize this system.

Exogenous GTP at a concentration of 10 μM resulted in only a 19% stimulation of AC with subsequent time-dependent inhibition, as was previously described in the

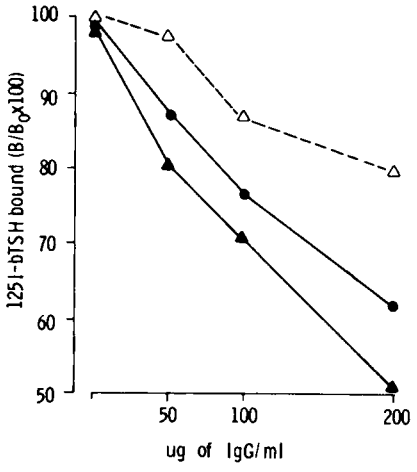

Fig. 2. Inhibition of ^{125}I-b TSH binding to thyroid membranes by anti-id. The effect of NRIgG (△) and of absorbed anti-id IgG on ^{125}I-b TSH binding to human (●) and porcine (▲) thyroid membranes were studied. The inhibition of ^{125}I-b TSH binding was more pronounced for the porcine membrane preparations, although they bound half the amount of radiolabeled thyrotropin as human membranes.

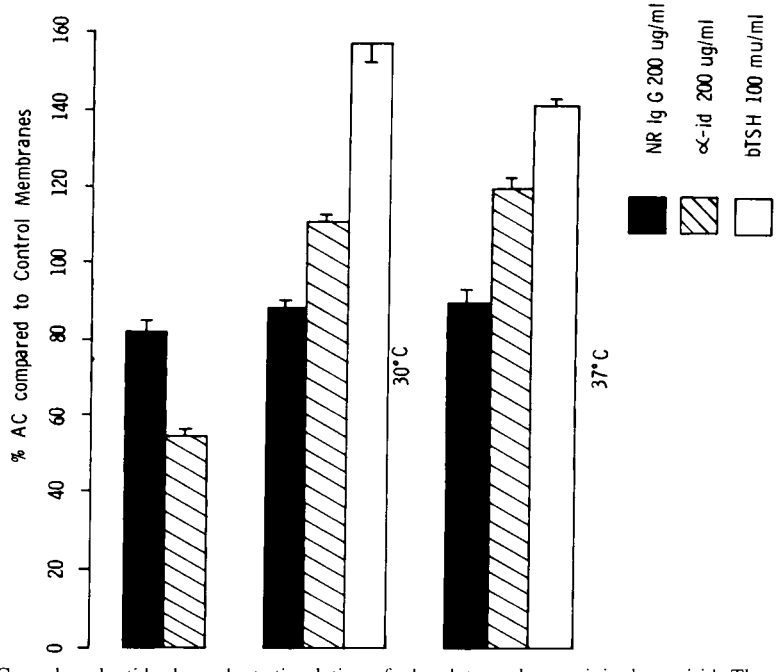

Fig. 3. Guanyl nucleotide-dependent stimulation of adenylate cyclase activity by anti-id. The upper panel shows relative AC activities at 30°C in the absence of Gpp(NH)p. Basal AC activity was 24 pM cAMP per mg protein per min. Because b TSH stimulated activity is not different from basal, it is not shown here. Anti-id caused significant inhibition of AC activity compared to NR IgG ($p < 0.05$). When 10 μM Gpp(NH)p was added to the incubate at 30°C basal AC activity increased to 113 pM cAMP per mg protein per min (middle panel). At 37°C AC activity was further enhanced to 304 pM cAMP per mg protein per min. At both these temperatures α-id significantly activated enzymatic activity compared to baseline ($p < 0.05$). The bars represent means ± SEM of six observations.

testicular membrane system [12]. In the presence of 10 μM Gpp(NH)p plasma membrane AC showed a sigmoidal dose response with maximal stimulation at 100 mU/ml b TSH and 50% stimulatory dose of 25 mU/ml; 50 mU/ml b TSH caused 91% of maximal AC activation. The stimulatory influence of Gpp(NH)p upon AC was dose-dependent and was linear over a 45-min period.

The influence of anti-id IgG upon porcine thyroid AC was tested under different conditions (Fig. 3). At 30°C anti-id reduced basal AC activity (by 32%) beyond that seen for NR IgG. With the inclusion of Gpp(NH)p in the assay mixture the antibody was, instead, stimulating (21%) compared to an equal amount of NR IgG. Increase (41%) in basal AC activity was induced by anti-id when the assay was performed at 37°C in the presence of Gpp(NH)p; in the absence of the guanyl nucleotide, no influence ($> 5\%$) on basal activity was seen. At a concentration of 200 μg/ml anti-id, the degree of AC stimulation was equivalent to 40 mU/ml of b TSH.

Neither NR IgG nor anti-id influenced the activation of AC by 10 mM sodium flouride that is thought to activate the enzyme by acting at guanyl nucleotide binding protein [13].

When incubated with dispersed thyroid cells for 10 min, anti-id caused a decrease in radioiodine uptake whereas incubation for 4 hr stimulated uptake (Fig. 4). In both respects the behaviour of the antibody is similar to that of TSH, whose initial effect is to increase the exit rate constant for iodide from the follicular cells transiently and only later to increase the entrance rate constant [14, 15].

DISCUSSION

Within the restriction imposed by the structure of the TSH molecule, we have made an anti-idiotypic antibody that acts as an agonist at the hormone receptor. This conclusion was reached through the following observations: (1) Anti-id caused a dose-dependent inhibition of ^{125}I b TSH binding to thyroid membranes. (2) The binding of ^{125}I anti-id to thyroid membranes was inhibited by bovine TSH in a dose-dependent manner. Because not all anti-id molecules are also specific for TSH binding, only two-thirds of ^{125}I anti-id are displaced by bovine TSH. This phenomenon reflects the fact that not all anti-idiotypic antibodies are directed against the antigen binding site [17]. (3) Anti-id is able to activate adenylate cyclase on thyroid plasma membranes through a guanyl-nucleotide dependent process (ie, through the occupancy of TSH receptor [13]). The antibody was further shown to: (4) Increase the entrance rate of ^{131}I into culture thyroid cells and (5) to cause this culture cells to organize into follicular structures.

The last two attributes of the antibody are characteristics of TSH action on thyroid cells and are a consequence of adenylate cyclase activation in as much as they are cyclic AMP dependent.

It is possible to conclude that anti-id, which bears spatial similarities to TSH, is homologous in action to the thyroid stimulating antibody (TSAb) of Graves disease. We cannot, however, exclude the possibility that TSAb preparations contain immunoglobulins directed against membrane structures adjacent to the receptor and that these may contribute to the TSAb biologic activity. It is of interest, however, that some of the unexpected influences of some thyroid stimulating antibodies, for example, the inhibition of accumulation of cyclic AMP [18], may also be reproduced by this antibody whose specificity is restricted to the receptor.

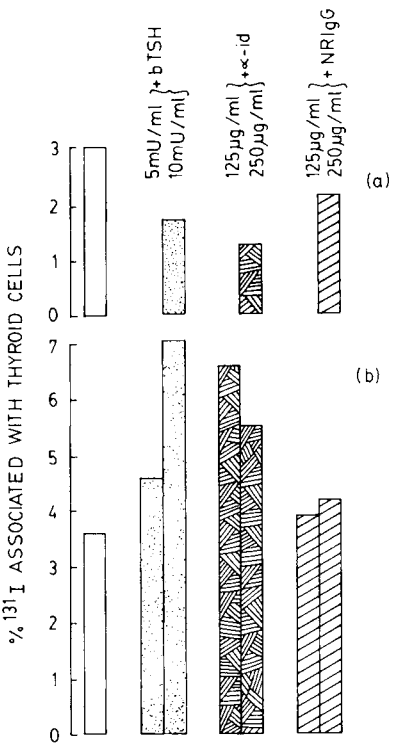

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Fig. 4. The influence of anti-idiotypic antibody on uptake of radioiodine by porcine thyrocytes. a) Radioiodine uptake was studied after incubation of thyroid cells with b TSH, NR IgG, or anti-id for 10 min (10^5 cpm). Na ^{131}I were then added and incubation continued for a further 10 min. Counts associated with pelleted cells were expressed as percentage of total counts added. Only the higher dosage of the experimental agents were used in these experiments. Both b TSH and anti-id caused a *decrease* in th ^{131}I taken up by thyrocytes. (b) The percentage of radioiodine associated with thyrocytes 4 hr after incubation with b TSH, NR IgG, or anti-id. One hundred and twenty-five μg/ml of anti-id caused a significant stimulation of ^{131}I uptake ($p < 0.05$). The higher concentration (250 μg/ml) caused less stimulation, raising the possibility that the anti-id preparation contains in lesser relative concentration an antibody species which inhibits iodine concentration.

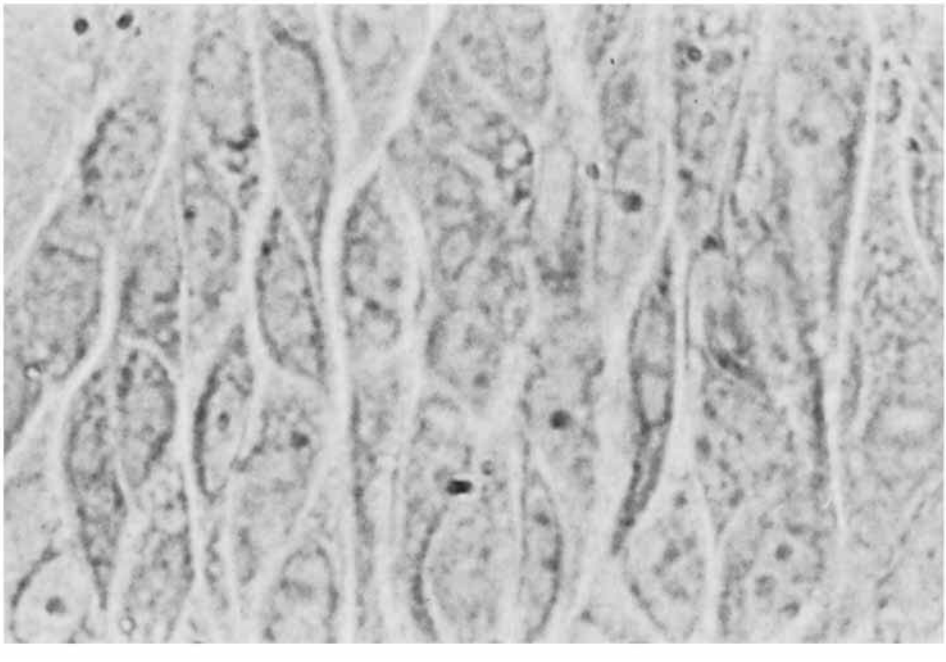

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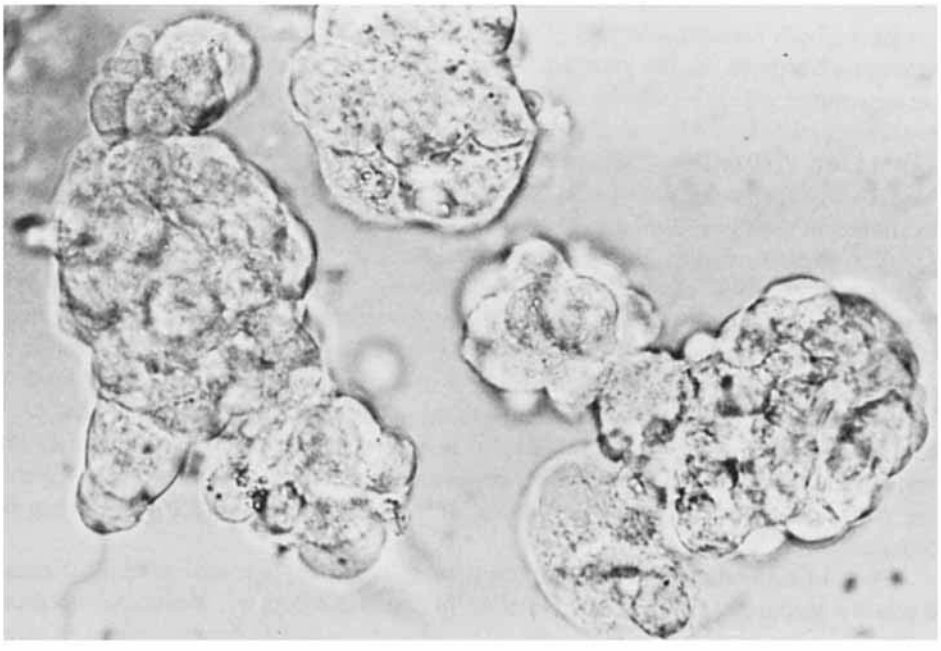
Fig. 5. Influence of thyrotropin, anti-id, and NR IgG on the follicular organization of cultured thyrocytes. (a) Cells cultured in absence of stimulator (in presence of NR IgG). Note monolayer disposition and fibroblast-like shape of cells. (b) Cells cultured in presence of b TSH. Note the three dimensional follicle-like structures. (c) Cells cultured in presence of anti-id. Cells have adopted the three dimensional follicle-like structure similar to the one obtained with b TSH. All photographs were taken on the seventh day of culture under same magnification (×520).



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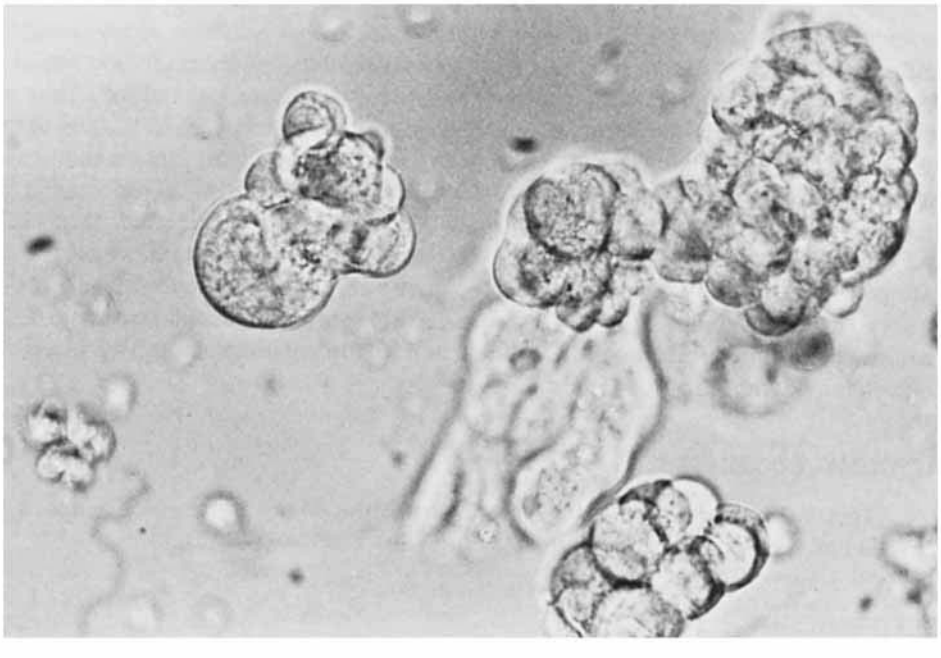

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Indeed, somatic cell hybridization of myeloma cells with splenic leucocytes from mice immunized with TSH receptor gave rise to monoclonal antibodies that were exclusively antagonistic [19]. This finding suggests that the interaction involves dominant epitope(s) on the receptor. The polyclonal anti-id preparation described here apparently comprise similar antibodies that inhibit adenylate cyclase activity and the accumulation of cAMP in thyroid cell preparations under appropriate conditions (Fig. 3) [20]. Presumably, antibodies against other epitopes present in the anti-id IgG are necessary for activation of cyclase either alone or when cross-linked to antibody specificites homologous to the monoclonal antibodies.

The present results further raise the distinct possibility that, at least in some patients with Graves disease, thyroid stimulating antibody may be an auto-anti-idiotypic antibody against TSH. Biro [21] has shown recently that the sera of healthy individuals and patients with Graves disease have a natural γ-globulin capable of specifically binding TSH. It is conceivable that in some patients with Graves disease, auto-anti-idiotypic antibody against this natural antibody may arise and that such antibodies may be agonistic at the receptor level. Whether or not thyroid stimulating antibodies arise naturally in the manner postulated, this work demonstrates the relative ease with which anti-idiotypic antibodies with thyroid stimulatory capacity may be produced.

Anti-id antibodies with antireceptor activity represent potential reagents that can be readily prepared for use in the isolation of specific receptors. Hormone receptors are found in low concentrations on target tissues necessitating the processing of large amounts of tissue and the isolation of receptor through multi-step processes with usually rather limited yield. An anti-id antibody production would substantially amplify on the amount of first antibody (id) produced as a result of immunization with ligands, many of which are available in purified forms. The question must, however, arise whether all anti-id antibodies against ligands will also be anti-receptor antibodies for that ligand. Theoretically, only when the immunogenic epitope of a ligand corresponds to the binding site of the hormone, will an anti-antibody have an antireceptor activity; if the immunogenic determinant is not involved in binding to the receptor, an anti-id is not expected to have an anti-receptor activity. Interestingly, the anti-id antibodies with antireceptor activity raised to date cover a wide range of ligands: insulin (a 6000-dalton polypeptide) [22] a beta-adrenergic antagonist [23], and now TSH (a 28,000 heterodimeric glycoprotein). The ease with which anti-id antibodies with activity against the corresponding receptors may be raised against a variety of ligands is remarkable. It is conceivable that this mechanism may be of importance in the mediation and the fluctuation of immunological activity in antireceptor antibody diseases.

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