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Immunology

Monoclonal human thyroid cell line GEJ expressing human thyrotropin receptors

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ABSTRACT **By using the hybridoma technology, a monoclonal human thyroid cell line was obtained by fusing fresh normal human thyroid cells with a human lymphoblastoid cell line. The resultant cell line, called GEJ, has been selected for its expression of thyrotropin (TSH) receptors and has morphological and functional characteristics of normal human thyroid cells. In the presence or absence of human TSH, the GEJ cell line accumulates iodide, produces thyroid hormones, expresses thyroid membrane antigens, and binds ≈600 molecules of TSH per cell. The binding site for TSH has all the characteristics of a specific receptor (i.e., temperature and time dependencies, dissociation of bound TSH only by high amounts of TSH, and a glycoprotein nature). Scatchard analysis described a curvilinear graph with two dissociation constants (K_d = 0.12 × 10^{-9} M and 1.6 × 10^{-9} M) with, respectively, 1.2 × 10^3 and 7.2 × 10^3 binding sites per cell. This human thyroid cell line that expresses TSH receptors could be a useful tool for the study of human thyroid disorders.**

Because of its potential importance in the pathogenesis of thyroid diseases, thyrotropin receptor (TSH-R) has long been the focus of multiple investigations. Although the receptor binding features have been studied with either cultured thyroid cells (1), thyroid homogenates (2), or thyroid plasma membranes, the molecular characteristics (3) concerning the structure and pathological relevance (4) of this receptor are largely dependent on the availability of fresh normal human thyroid tissue in large quantities. To overcome this problem, we have used the hybridoma technology (5) and fused fresh normal human thyroid cells with a human lymphoblastoid cell line. The aim of the present study was to establish a human cell line with morphological and physiological features of a human thyroid cell in order to investigate the pathology of thyroid disorders. This hybridization technique provided a readily available cell line. The availability of such a tool allows biochemical and immunological studies using the same material throughout an extensive period of experimentation.

MATERIALS AND METHODS

Human Thyroid Cells. Cells were obtained from human paranodal tissue from thyroids obtained after lobectomy was performed for benign nodules. Tissue samples were dissected and minced at 4°C in RPMI-1640 culture medium (GIBCO, Paisley, Scotland, U.K.) supplemented with 10% fetal calf serum. The suspension was incubated with collagenase dispase at 5 mg/ml (Boehringer Mannheim) in a shaking water bath for 30 min at 37°C.

The cells were then washed in RPMI-1640 medium supplemented with penicillin (100 units/ml)/streptomycin (100 μg/ml)/fungizone (2.5 μg/ml)/1% glutamine/10% fetal calf serum (complete medium).

Lymphoid Cells. The human GM1500 6TG-A12 lymphoblastoid cell line deficient in hypoxanthine phosphoribosyltransferase activity (5) was used. These cells were grown in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal calf serum.

Cell Hybridization. GM1500 6TG-A12 cells (0.6 × 10^6) were fused with 3 × 10^6 thyroid cells in the presence of polyethylene glycol (5). After fusion, cells were dispersed, incubated, and transferred into wells of Microtest II plates (Falcon 3042) and fed by using selective medium as described (6). From 25 to 30 days after fusion, the cells were cloned by limiting dilution analysis in Microtest II culture dishes seeded with 0.5 cells per well. Two months later, 15 clones were tested for ^{125}I-labeled TSH (^{125}I-TSH) binding. One of these was found to be positive in binding assays, and we call it GEJ.

Characterization of a Specific Receptor for TSH. Binding studies were performed in 0.1 ml of RPMI-1640 medium supplemented with 1% Hepes and 1 μM phenylmethylsulfonyl fluoride (Sigma). Triplicates of 1 × 10^6 GEJ cells were incubated with 10 ng of human ^{125}I-TSH (New England Nuclear; 55 μCi/μg; 5 × 10^5 cpm/10^6 cells; 1 Ci = 37 GBq) in the presence or absence of unlabeled human TSH (Boehringer Mannheim) up to a 1000-fold excess. Background binding studies were performed at the same time by incubating 1 × 10^6 lymphoblastoid cells with 10 ng of ^{125}I-TSH. At the end of the incubation, the individual samples of each triplicate of the GEJ cell line were layered onto 0.3 ml of a 25% (vol/vol) sucrose solution and centrifuged at 8000 × *g* for 90 sec. The tubes were cut and the pellets and the supernatants were assessed for bound and free ^{125}I-TSH.

Specifically bound radioactivity was defined as the difference between radioactivity bound in the absence and in the presence of unlabeled hormone (10 μg). The results are expressed as the mean number of ^{125}I-TSH molecules per cell.

Morphological Studies. For electron microscopic studies, 1 × 10^6 GEJ cells were cultured with or without human TSH (100 milliunits/ml). On day 7 of culture, GEJ cells were fixed at 4°C for 1 hr with 2.5% glutaraldehyde in 2 ml of 0.2 M cacodylate buffer (pH, 7.2) and washed for 1 hr in the same buffer. Cells were then fixed with osmium in cacodylate buffer and preparations were embedded in Epon after washing with Tris·HCl buffer. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined in a Siemens-Elmiskop 101 electron microscope at 80 kV.

Determination of Microsomal Antigens on GEJ Cells. A complement-dependent cytotoxicity assay was used in the presence of sheep anti-human microsomal antibodies (Wellcome Diagnostics). GEJ cells in microtest II plate cultures

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Abbreviations: TSH, human thyrotropin; TSH-R, TSH receptor; ^{125}I-TSH, ^{125}I-labeled TSH.

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were labeled for 18 hr at 37°C with 5000 cpm of ^{51}Cr (New England Nuclear). At the end of this incubation, labeled GEJ cells were washed twice with complete medium, and then 100 μl of diluted specific antibody was distributed into three wells for classical complement-dependent cytotoxicity. Simultaneously, cytotoxicities were determined on lymphoblastoid cells and on normal human thyroid cells as controls.

Measurement of ^{125}I Uptake by Cells. GEJ cells or GM1500 6TG-A12 cells (10×10^6) were incubated for 6 hr in 1 ml of Dulbecco's modified Eagle's medium containing 5 μCi of carrier-free $Na^{125}I$, and 1 mM unlabeled iodide, and 3 mM of 1-methyl 2-mercaptoimidazole. Incubations were at 37°C in a humidified atmosphere and were terminated by centrifugation. To determine the amount of cell-associated ^{125}I, 1 ml of 10% trichloroacetic acid was added to each tube for 20 min. ^{125}I uptake was expressed as the percentage total activity. Potassium perchlorate was added at the steady state. Reactions were terminated at different times by rapid centrifugation.

Isolation of Hormones from GEJ Cell Supernatant. GEJ cells (10×10^6) were cultured for 6 hr in 2 ml of culture medium with TSH and without fetal calf serum. Iodide uptake was initiated by adding 5 μCi of carrier-free $Na^{125}I$ (specific activity, 100 mCi/mmol). Incubations were at 37°C in a humidified atmosphere and were terminated by centrifugation at $150 \times g$ for 5 min. The soluble iodoproteins were purified from the radioactive supernatant as described (7). Briefly, protein hydrolysis was routinely performed with Pronase at a concentration of 3 mg/ml in 0.2 M Tris·HCl (pH, 8.0), 1-methyl 2-mercaptoimidazole (10 mM), and a few drops of toluene. Pronase hydrolysis lasted 12 hr at 37°C.

The iodo amino acids were fractionated on 1.5×20 cm Dowex 1 columns. After hydrolysate was applied, the columns were rinsed with 20 ml of water and eluted with 0.1 M HCl/butanol (3.3%, vol/vol). Two peaks were eluted sequentially. Hormones were eluted with 60% acetic acid. Iodide was retained by the column. In a second set of experiments, RIA for T3 and T4 detection in the GEJ cell supernatant harvested from 3-day-old cultures of 1×10^6 cells, in the presence or absence of TSH (100 milliunits/ml), was performed after Pronase hydrolysis. Specific human anti-T3 (ref. no. 79680) and anti-T4 (ref. no. 79690) antibodies (J. Dray, Unité de Radioimmunologie Analytique, Institut Pasteur, Paris), both with curve sensitivities of 30–300 pg per assay tube, were used.

Detection of Thyroglobulin in Cell Culture Supernatants. GEJ cells (100×10^6) were cultured with or without TSH (10 milliunits/ml). Culture supernatants that had not been changed were collected on days 1, 2, 3, 4, and 5, passed through 45-μm filters and dialyzed for 72 hr against 5 liters of phosphate-buffered saline diluted 1:100 (Eurobio, Paris), lyophilized, and resuspended in 0.5 ml of phosphate-buffered saline. A large molecule (600–700 kDa) was isolated from the supernatants by means of gel filtration on a Sepharose 6B column (1.5×90 cm; flow rate, 10 ml/hr; Pharmacia, Uppsala, Sweden) previously calibrated with human thyroid crude extract and equilibrated in phosphate-buffered saline (pH, 7.2). Protein determination was performed at 280 nm for each fraction collected. Fractions were pooled according to protein peaks. The thyroglobulin concentration was determined by an immunoradiometric assay (8) and the results are expressed as ng of thyroglobulin per ml of supernatant. The same procedure was followed using lymphoblastoid cells as controls.

RESULTS

Binding of ^{125}I-TSH to the GEJ Cell Line: Effect of Temperature and Time of Incubation. In the first set of experiments, 15 clones were tested for their ability to bind ^{125}I-TSH at 33°C for 30 min. Under these experimental conditions, 7 clones bound <100 ^{125}I-TSH molecules per cell, 8 clones bound between 100 and 500 ^{125}I-TSH molecules per cell, and the selected cell line (GEJ) bound 550 ± 70 molecules of ^{125}I-TSH per cell in three different experiments. The amount of ^{125}I-TSH molecules bound per cell is temperature and time dependent. The maximum binding occurred at 33°C after either 30 or 60 min, whereas approximately 60, 110, and 230 ^{125}I-TSH molecules were bound, respectively, per cell at 4°C, 22°C, and 37°C after equivalent incubation times. Under optimal experimental conditions (1×10^6 cells; 33°C), the effect of the time of incubation was studied (Fig. 1). When the GEJ cells were incubated with ^{125}I-TSH, a steady state was reached at 60 min and was maintained until 180 min. In the presence of a large excess of unlabeled TSH (10 μg), only a small percentage of ^{125}I-TSH was bound. To determine whether the receptors were degraded, GEJ cells were preincubated at 33°C for 0–120 min prior to the addition of labeled TSH for standard incubation. Neither specific nor nonspecific binding was altered by this preincubation. This result makes the release of soluble receptors during the binding period very unlikely.

Effect of Trypsin or Neuraminidase on Binding. Trypsin was used to investigate the capacity of the GEJ cells to regenerate TSH receptors after their destruction by proteolysis. No TSH binding was detected after a 15-min incubation with trypsin (10 or 40 μg/ml) followed by extensive washing. However, normal binding capacity was recovered after incubating GEJ cells in trypsin-free medium overnight at 37°C (Fig. 2). To determine whether this binding structure was a glycoprotein, we used neuraminidase (Behring, Marburg, F.R.G.), a glycolytic enzyme. No TSH binding was detected after a 60-min incubation with neuraminidase (0.02 units for 10^6 cells) followed by extensive washing. It must be noted that after treatment with this enzyme, ^{125}I-TSH binding was not recovered after overnight incubation at 37°C, as was reported for trypsin.

Scatchard Analysis. A saturation curve was obtained by incubating GEJ cells with ^{125}I-TSH (10 ng) and varying the concentration of unlabeled TSH (1 ng to 10 μg). Data were plotted according to Scatchard's method (9) and subjected to linear regression analysis. A curvilinear graph was obtained, allowing two hypotheses: either negative cooperativity or two independent binding sites. According to the latter hypothesis, values for the dissociation constant K_d were 0.12×10^{-9} M and 1.6×10^{-9} M with, respectively, 1.2×10^3 and 7.2×10^3 binding sites per cell (Fig. 3).

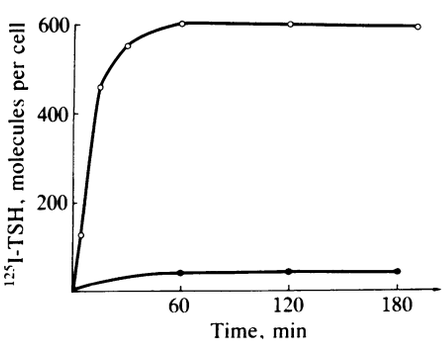

FIG. 1. Kinetics of TSH binding to GEJ cell line. Cultured cells (10^6 cells per 0.1 ml) were incubated at 33°C with 10 ng of ^{125}I-TSH with (●) or without (○) excess unlabeled TSH (10 μg). At times indicated, reaction was stopped by layering cells onto 0.3 ml of a 25% sucrose solution and centrifugation at $8000 \times g$. Radioactivity bound to the cells in the absence of unlabeled TSH was referred to as total binding and radioactivity bound in the presence of unlabeled TSH was referred to as nonspecific binding.

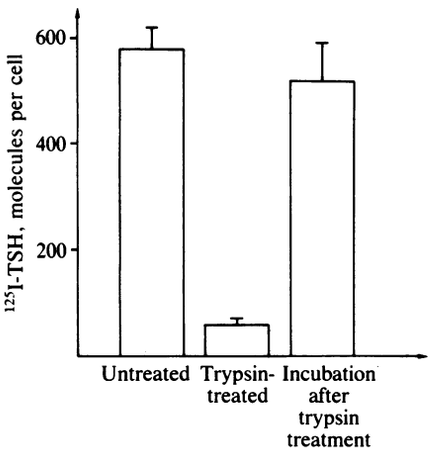

FIG. 2. Effects of trypsin treatment on TSH binding. GEJ cells (10^6) were treated with trypsin (40 μg/ml) at 37°C for 15 min. Soybean trypsin inhibitor (1 mg/ml) was added to stop the reaction and the cells were washed 3 times in complete medium and centrifuged at 600 × *g* for 10 min. The supernatant was discarded and the cells were resuspended under the initial conditions. Binding capacity was assayed as described, after overnight incubation at 37°C in a mixture of 5% CO_2/95% air. Cell viability was unaffected by this treatment Mean ± SEM of 3 determinations.

Reversibility and Specificity of Binding. When equilibrium was reached (33°C, 60 min), the addition of 10 μg of unlabeled TSH (1000 times the amount of labeled TSH) induced the dissociation of the bound ^{125}I-TSH in 180 min (Fig. 4). Partial inhibition was obtained 30 and 60 min after the addition of unlabeled TSH. The capacity of the GEJ cell line to bind other peptides, such as human luteinizing hormone and corticotropin (ACTH) was investigated using the methodology described above for TSH. Ten micrograms of luteinizing hormone, which shares a common subunit with TSH, inhibits 45% of the ^{125}I-TSH from binding to GEJ cells, while 10 μg of corticotropin, a peptide hormone not related to TSH, did not show any significant inhibition of ^{125}I-TSH binding (Fig. 5).

Morphological Studies. GEJ cells grown in the absence of TSH showed polarity with numerous vesicles at the apical pole (Fig. 6). When cultured in the presence of TSH, although no reassociation into follicles was observed, GEJ cells looked hyperplastic with a well developed Golgi apparatus and with a mitotic index twice as high.

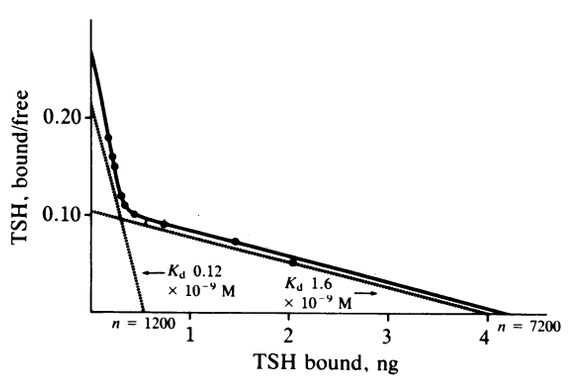

FIG. 3. Evaluation of the number of sites per cell at 33°C and dissociation constants. GEJ cells (10^6) were incubated with ^{125}I-TSH (10 ng) and various concentrations of unlabeled TSH. Nonspecific binding was determined in the presence of excess unlabeled TSH (10 μg). Ratio of bound to free ^{125}I-TSH is plotted according to Scatchard's method as function of specifically bound TSH expressed in ng. *n*, Binding sites per cell.

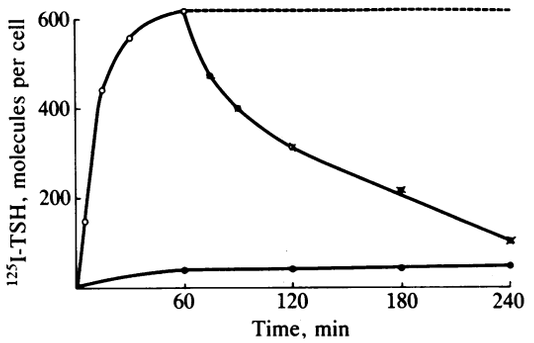

FIG. 4. Dissociation of labeled TSH from its receptors. GEJ cells (10^6) were incubated at 33°C with ^{125}I-TSH (10 ng) with (●) or without (○) unlabeled TSH (10 μg). Unlabeled TSH (⊗) was added to the tube containing ^{125}I-TSH alone after a 60-min incubation.

Detection of Microsomal Antigens. In four consecutive experiments, the mean cytotoxicity index for the GEJ cell line was 106 ± 16, while that for GM1500 6TG-A12 was negative.

Iodide Accumulation by GEJ Cells. Iodide accumulation was determined and compared to that of GM1500 6TG-A12 cells. GEJ cells accumulate iodide, reaching a steady-state level within 30 min (Fig. 7). The addition of perchlorate at the steady state led to a rapid loss of >90% of the accumulated iodide, GM1500 6TG-A12 cells amassed <10% of the amount accumulated by GEJ cells.

Production of Thyroid Hormones by GEJ Cells. Two milliliters of supernatant obtained from a 6-hr culture of 10 × 10^6 GEJ cells with TSH was fractionated on Dowex 1 columns. As shown in Fig. 8, after elution with 0.1 M HCl/butanol, two peaks were obtained containing monoiodotyrosine and diiodotyrosine. After elution with 60% acetic acid, a third peak was obtained that contained the thyroid hormones. The nature of the three hormone peaks was verified by paper chromatography. In other experiments, we used an RIA for T3 and T4 detection. After a 3-day culture of 1 × 10^6 GEJ cells in the presence or absence of TSH, T3 was detected in the supernatant at 0.1 ng/ml. After Pronase hydrolysis of these supernatants, 0.7 ng of T3 per ml was found for cells cultured in the presence of TSH and 0.6 ng/ml was found for cells cultured in the absence of TSH. For T4 detection without Pronase hydrolysis and in the presence of TSH, GEJ cells secreted 2.6 ng/ml, while they produced 2.2 ng/ml in absence of TSH. After Pronase hydrolysis, T4 secretion was, respectively, 3.2 ng/ml and 2.6 ng/ml for cells cultured in the presence or absence of TSH. It must be emphasized that in GM1500 6TG-A12 cell supernatants, no T3 was de-

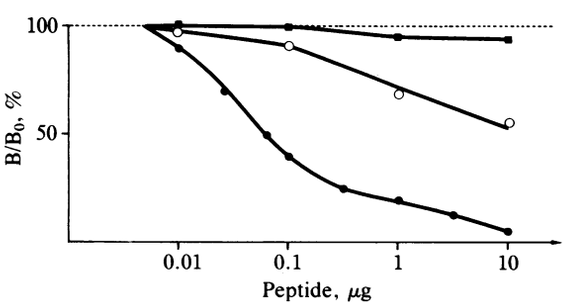

FIG. 5. Inhibition of binding of ^{125}I-TSH by luteinizing hormone and corticotropin. In 0.1 ml, 10^6 GEJ cells were incubated for 60 min at 33°C with ^{125}I-TSH (10 ng) in the presence of peptide hormone in the concentrations indicated. ●, TSH; ■, corticotropin; ○, luteinizing hormone. Results are expressed as percentage of ^{125}I-TSH bound in the presence (B) and in the absence (B_0) of unlabeled peptide.

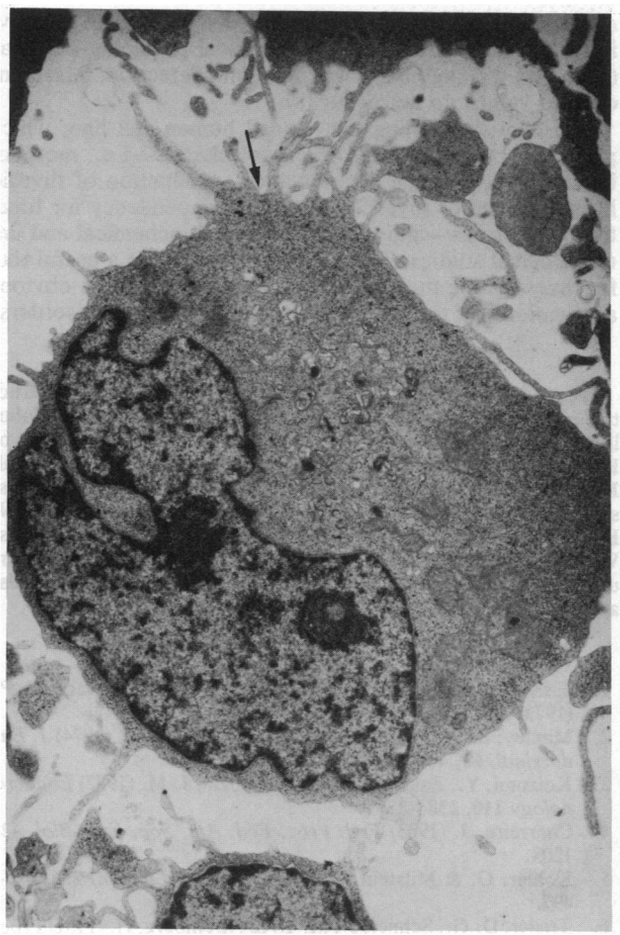

FIG. 6. GEJ cells cultured in medium without TSH-isolated cells are polarized. Polarization is shown by excentric nucleus and localized membrane expansions (→). (×14,300.)

tectable and T4 levels were always <1 ng/ml. From these results, values obtained for complete medium alone were subtracted.

Detection of Thyroglobulin in GEJ Cell Supernatants. Using an immunoradiometric assay (8), increasing amounts of thyroglobulin were found in conjunction with the prolonged duration of GEJ cell culture (Fig. 9). The presence of TSH in the culture medium led to a higher production of thyroglobulin regardless of the duration of culture. In contrast, thyroglobulin was never detectable in GM1500 6TG-A12 cell supernatants. It should be pointed out that culture supernatants were not changed.

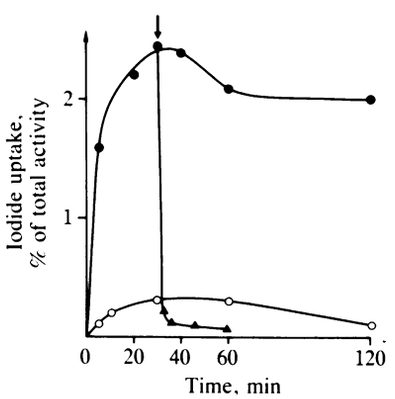

FIG. 7. Time course of iodide accumulation in GEJ (●) and GM1500 6TG-A12 (○) cells. As indicated by arrow, 1 mM $KClO_4$ (▲) was added to triplicate tubes. Reactions were carried out at 33°C and ended at the indicated times.

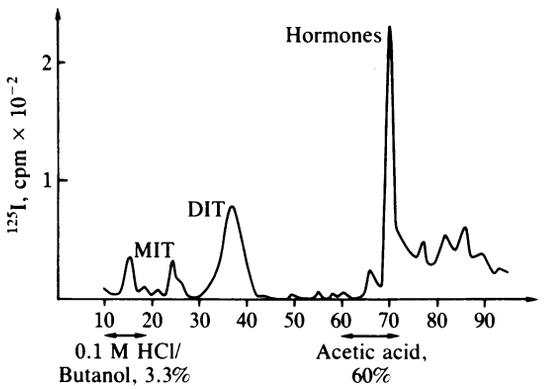

FIG. 8. Elution profile obtained by Dowex 1 chromatography of GEJ cell supernatants hydrolyzed with Pronase. The nature of monoiodotyrosine (MIT), diiodotyrosine (DIT), and hormones were verified by paper chromatography. The first peak was eluted in the void volume.

DISCUSSION

To easily study the human TSH-R and its relevance in some pathological states, as well as the biochemical features of human thyroid cells, we have used the hybridoma technology. The thyroid cell line created, GEJ, resulted from the fusion of normal human thyroid cells with a human lymphoblastoid cell line. GEJ cells present most of the morphological and functional characteristics of normally differentiated human thyroid cells. By using an immunoenzymatic assay, the hybrid nature of this cell line is demonstrated by the detection in GEJ culture supernatant of IgG of the same isotype (IgG2 κ; data not shown), as that found in the lymphoid cell culture supernatant. GEJ cell line was selected on the basis of TSH-R presence. It must be pointed out that the GEJ cell line has now been cultured and regularly subcloned in our laboratory for 18 months without loss of its characteristics.

Our data show that TSH binding to the GEJ cells is a saturable, specific, and temperature-dependent process. In this system, a low temperature can alter thermodynamically dependent parameters of association and dissociation, and it modifies the ligands or the membrane environment of the receptors. The better fixation of ^{125}I-TSH to GEJ cells at 33°C than at 37°C can be explained by the partial degradation that occurs at 37°C (10, 11). The binding specificity of ^{125}I-

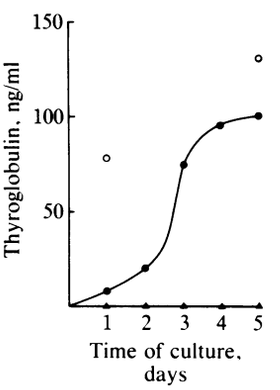

FIG. 9. Production of thyroglobulin by GM1500 6TG-A12 cells (▲), GEJ cells cultured without TSH (●), or with TSH (○) after different periods of culture. Results are expressed as ng per ml of supernatant.

TSH to the cell line is assessed by the lack of displacement observed in the presence of corticotropin, an unrelated peptidic hormone, while luteinizing hormone, which shares a subunit with TSH, induces a partial displacement.

The sensitivity of the cellular receptors to destruction by trypsin or neuraminidase is quite remarkable. This sensitivity to proteolytic and glycolytic enzyme is in agreement with the glycoprotein nature of the receptor (10) normally present on thyroid cells. Our results demonstrate that the interaction between ^{125}I-TSH and the TSH-R on GEJ cells is a complex process. It is characterized by a nonlinear Scatchard plot, which can be interpreted as initially reported for insulin—by negative cooperativity between sites (12)—or by the presence of two sites, one with a high affinity and a low capacity, and the second with a low affinity and a high capacity. This curvilinear graph has already been described for bovine (10) and human TSH-R (13). Recently, Drummond *et al.* (14) showed that this non-linear Scatchard plot results from at least two distinct binding sites rather than negative cooperativity between sites. The possibility of two cell populations with two different sites is made unlikely by the assumed monoclonal nature of the long-term cell line used in this study. The reversibility of ^{125}I-TSH binding to GEJ cells is assessed by the addition of unlabeled TSH (1000-fold excess), which produces a total dissociation of cell-bound ^{125}I-TSH within 180 min.

Morphological studies using electron microscopic methods show that GEJ cells display a polarity similar to human thyroid cells. They are sensitive to the trophic action of TSH as assessed by the well developed Golgi apparatus and the increased mitotic index. Nevertheless, they are unable to reassociate into follicles. Lastly, these cells express microsomal antigens specific to thyroid cell surfaces, to a degree comparable to that of normal human thyroid cells. These results must be interpreted with caution, because the anti-microsomal antibodies used are polyclonal. In addition to these morphological features, GEJ cells have numerous functional hallmarks of normal thyroid cells. These cells are able to accumulate iodide; this accumulation is an active and not a diffusional process, as shown by the effect of perchlorate. Moreover, the elution profile obtained by Dowex 1 chromatography of iodocompounds, provides evidence that these cells can also produce hormones and their precursors (mono- and diiodotyrosine). The small quantity of iodocompounds produced may be explained by the culture of these cells without serum. The fact that we found, either by anion exchange chromatography or by paper chromatography, greater quantities of hormones than mono- and diiodotyrosine needs to be further investigated. Thyroid hormone production is also detected using a highly sensitive RIA. This production is slightly enhanced by TSH addition. Pronase hydrolysis of supernatants increases the level of hormone detected; this effect means that culture supernatants contain an iodinated protein. In line with this, these cells are able to produce thyroglobulin, this thyroglobulin production increases during 5 days of culture and displays a high sensitivity to TSH, in agreement with classical physiological ideas (15). With this method of thyroglobulin detection, falsely increased values do not occur (8).

Our data concerning this cloned human cell line, which possesses numerous thyroid characteristics—i.e., morphological aspect, iodide accumulation, production of thyroid hormones and of thyroglobulin, TSH dependency for function and growth—allow us to envisage biochemical and immunological studies. This cell line seems to be a useful tool for investigating the nature of human TSH-R, with obvious applications for the study of autoimmune thyroid disorders.

We are deeply indebted to Professor J. C. Savoie for his instructive comments and encouragement, and for measuring thyroglobulin. We also acknowledge the excellent technical assistance of Eliette Lallemand and Anne-Marie Athouel-Haon and we thank Jean-Marie Pleau and Jean Salamero for useful suggestions, discussions, and constant encouragement. We are grateful to Doctor N. Etling (Hôpital Necker, Paris) for RIA T3 and T4 determinations. We are indebted to Dr. Gandon and to Dr. J. Andre (Clinique Chirurgicale de la Porte de Choisy, Paris) for providing human thyroids, and to Janet Jacobson for her editorial assistance.

1. Lissitzky, S., Fayet, G., Verrier, B., Hennen, C. & Jacquet, P. (1973) *FEBS Lett.* **29**, 20.
2. Manley, S. W., Bourke, J. R. & Hawken, R. W. (1974) *J. Endocrinol.* **61**, 419–436.
3. Koizumi, Y., Zakarija, M. & McKenzie, J. M. (1982) *Endocrinology* **110**, 1381–1391.
4. Charreire, J. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42**, 1203.
5. Kohler, G. & Milstein, C. (1975) *Nature (London)* **256**, 495–497.
6. Trisler, D. G., Schneider, M. D. & Niremberg, H. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2145–2149.
7. Savoie, J. C., Thomopoulos, P. & Savoie, F. (1973) *J. Clin. Invest.* **52**, 106–125.
8. Bayer, M. F. & Kriss, J. P. (1979) *J. Clin. Endocrinol. Metab.* **49**, 557–564.
9. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672.
10. Amir, S. M., Goldfine, I. D. & Ingbar, S. H. (1976) *J. Biol. Chem.* **251**, 4693–4699.
11. Kohn, L. D. (1978) in *Receptors and Recognition*, eds. Quatrecasas, P. & Greaves, M. F. (Chapman & Hall, London), Ser. A., Vol. 5, pp. 134–212.
12. De Meyts, P., Roth, J., Neville, D. M., Cravin, J. R. & Lesniack, M. A. (1973) *Biochem. Biophys. Res. Commun.* **55**, 154–161.
13. Lissitzky, S. (1979) in *Autoimmune Aspects of Endocrine Disorders*, eds. Pinchera, A., Doniach, D., Fenzi, C. F. & Baschieri, L. (Academic, New York), pp. 73–81.
14. Drummond, R. W., McQuade, R., Grünwald, R., Thomas, C. G., Jr., & Nayfeh, S. N. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2202–2206.
15. Sterling, K. & Lazarus, J. H. (1977) *Annu. Rev. Physiol.* **39**, 349–371.