# THYROID AUTOIMMUNITY

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# THYROTROPHIN AND GROWTH PROMOTING IMMUNOGLOBULIN(TGI) OF FRTL-5 CELLS HAVE NO GROWTH STIMULATING ACTIVITY ON HUMAN THYROID EPITHELIAL CELL CULTURES

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## INTRODUCTION

When growth promoting activity on thyroid cells was discovered in immunoglobulin fractions (TGI) from patients with goitrous Graves' disease (GD), an intriguing concept to explain goitre formation was created (1). Indeed, TGI-like activity subsequently was described in patients with non-toxic (NTG) or recurrent goitres (2,3,4). Recently however, some doubts were cast on this concept for goitre formation, since TGI activity could not be found in highly purified IgG fractions and growth promoting activity in IgG fractions prepared by ammonsulfate precipitation was attributed to contaminations with epidermal growth factor (EGF) (5). Moreover, TGI has not yet been convincingly detected with assay systems using human thyroid epithial cells (TEC).

Our own interest to reassess TGI activities was triggered by the inability to find TGI in serum from patients with euthyroid or recurrent goitres (not shown). We therefore investigated the growth promoting activities of TGI samples, TSH and EGF on human TEC and on rat FRTL-5 cells. In both assay systems tritriated thymidin ($^3$HTdR) uptake, glucose-6-phosphate dehydrogenase (G-6-PD) and adenylcyclase (cAMP) were measured.

## METHODS

TECs were established from thyroid tissue obtained at operation of patients with GD or NTG. After separating TECs from debris and erythrocytes by density centrifugarion cells were plated in Iscove medium supplemented with insulin, transferrin, hydrocortisone, somastatin, and gly-his-lys tripeptide in 0.5% FCS (5H medium) (6). 20 x $10^3$ cells 1 per well of 96 well Microtiter flat bottom plates were growing in semi follicle-like structures (domes) displaying "right side out" polarity. The differentiated function of TECs in cultures was verified by their ability to secrete thyroglobulin and to express microsomal(M) antigen on TSH stimulation.

The rat FRTL-5 cells were grown in Coon's 5H medium plus 1 mU bTSH/ml. Before these cells were used for TGI assays TSH was withdrawn for 5 days. Both TECs and FRTL-5 cells were used for $^{3}$HTdR uptake in 96 well plates after cultures reached 2/3 confluency. The supernatants of cultures were used for measuring cAMP stimulation (7) of cells. Cell cultures were preincubated for 48 h with IgGs (0.1mg/ml ),TSH (10-$10^{4}$µU/ml) or EGF (0.1- 5 ng/ml). In order to measure the 24h $^{3}$HTdR uptake cells were washed, detached from plastic bottoms by trypsin digestion and harvested on cellulose acetate filter with a cell harvester using 10% tri- chlor-acetic acid, and 96% cold ethanol. Results were calculated as stimulation indices(8). The G-6-PD was measured kinetically (9) using cells grown and preincubated in 16 well Costar plates. The G-6-PD activity was expressed as % over control cultures. Three IgGs from patients with GD were used for the study. They were positive for thyroid stimulating antibodies (TSab) and showed growth promoting activity in the $^{3}$HTdR uptake assay of FRTL-5 cells. As a normal control a pool of IgGs from healthy individuals of the laboratory staff was used. All IgGs were prepared from serum by ionexchangechromotographie on Affi-Gel- Blue and concentrated by Minicon- ultrafiltration chambers. All IgG samples were adjusted to 1mg/ml and stored in liquid nitrogen until use.

## MATERIALS

Collagenase (Dispase II) was from Boehringer, Mannheim, FRG. Fetal calf serum (FCS), Iscove medium and additives were from Biochrom, West Berlin. Coon's modified medium was from GIBCO, Europe, Karlsruhe, FRG. TSH (Thyrostimulin) was from Organon, Munich, FRG. EGF and all hormone additives were from Sigma Chemie, Deisenhofen, FRG. Tritiated thymidin and the cAMP binding-protein assay were from NEN, Dreieich, FRG. Affi-Gel- Blue was from BioRad Lab., Munich, FRG. The FRTL-5 rat cells were kindly provided by Dr. L. Kohn, Bethesda, USA.

## RESULTS

By stimulating FRTL-5 rat cells with TSH (Fig.1a) the growth promoting activity is apparently mediated by adenylcyclase activation. In addition, the $^{3}$HTdR uptake is paralleled by the activation of G-6-PD. Human TECs display a different pattern of TSH stimulation (Fig.1b). Although TSH is stimulating the adenyl-cyclase, HTdR uptake as well as G-6-PD stay flat.

A similar result is obtained with IgG preparations from GD-patients (n=3): With FRTL-5 cells the $^{3}$HTdR uptake increases by 2.8 - 5.9 times accompanied by an cAMP increase of 200-600% (Fig. 2). In contrast, human TECs after incubation with IgGs (GD) do not incorporate significantly more thymidin than control IgGs, although cAMP increases by 120-400% compared to pooled normal IgG.EGF served as a control for the proliferation assay with human TECs. Thymidin uptake was increased by 2.5-7.1 fold (n=4), while FRTL-5 cells of course could not be stimulated by EGF.

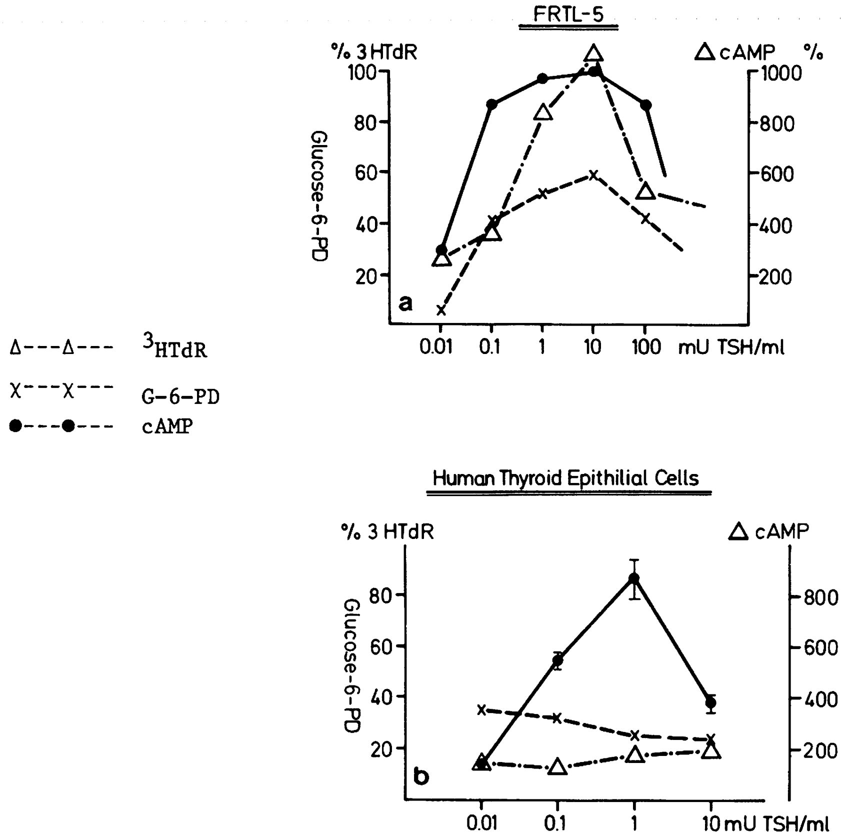


FIGURE 1.

Growth and cAMP stimulation by bTSH

DISCUSSION

Various experimental assay systems (1,2,3,4) using thyroid cells from different species (1,3,4) have been employed to demonstrate thyroid growth stimulators in IgG preparations from patients with auto-immune and non-immune goitrous thyroid disorders (1,2,4). Both, the assay systems and the origin of thyroid cells have rather contributed to confusion than to clarify thyroid cell growth in vitro and goitre formation in vivo. This is particularly true for the human thyroid, where the mechanisms of proliferation control are not yet understood.

While TSH mediates growth stimulation through a cAMP signal in dog and rat cells (10,11), no such effect is observed in pig and sheep thyroid cells (12,13). We here confirm that TSH does not induce proliferation in human TECs, although it activates adenylcyclase (Fig. 1b)(14,15,16). In contrast, FRTL5 cells stimulated with TSH showed increased cAMP thymidine uptake and G-6-PD activation, which is indirectly linked to DNA-synthesis (Fig.1a) (17). Since in FRTL-5 cells growth is mediated by adenyl cyclase activation , it makes FRTL-5 cells a dubious tool to measure TGI activities from hyperthyroid GD-patients. Characteristically, these patients mostly have cAMP stimulating Tsab. Thus,with TSab positive GD-IgGs, the FRTL-5 cell system should always detect growth stimulation. But even the FRTL-5 cell system has been reported to detect selectively TGI in IgGs of goitrous GD-patients (3).This might be due to the insensitivity of assay systems employed. It does , however, still allow an adenylcyclase independent growth stimulator acting on FRTL-5 cells.

These growth promoting IgGs derive from humans and supposedly stimulate goitre formation in vivo. One should therefore assume that these stimulators would promote growth of human TECs in vitro, if they do react directly on the thyroid cells and if TECs still have differentiated characteristics in vitro. TECs in our hands, were almost free of fibroblasts (5H medium). They displayed M-antigen on TSH and TSab stimulation and they synthesized thyroglobulin. With our, admittedly, small number of IgGs from goitrous, hyperthyroid GD-patients, we could not detect growth promoting activity (Fig. 2), although the same IgGs stimulated $^3$HTdR uptake in FRTL-5 cells.

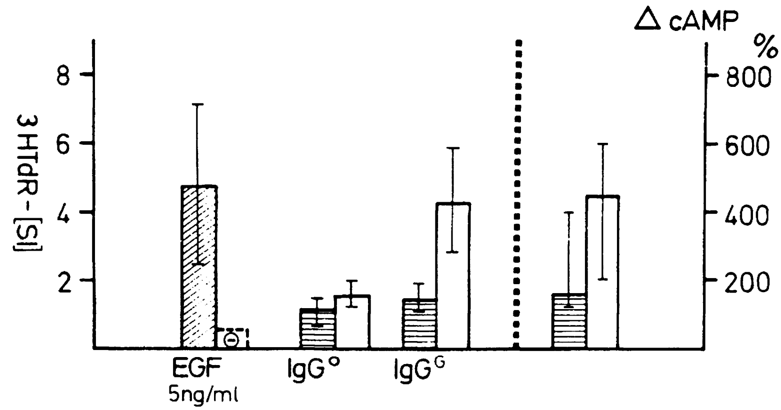


FIGURE 2.

Growth promoting and stimulating activities of IgG from GD-patients(IgG$^G$),IgG from normals(IgG$^\emptyset$) and of EGF.

-- FRTL-5 cells -- Human TECs

The situation in euthyroid and recurrent goiters appears even more conflicting, since growth promoting activity in IgG prepared by ammoniumsulfate precipitation was attributed to EGF contaminations. We were not able so far to demonstrate stimulation of cell growth with IgGs prepared by ionexchange-chromatography using the HdR uptake of human TECs.

In conclusion, future work on growth promoting activities in human IgGs , should not only define better the acting agent but also the mechanism by which cell proliferation is modulated.

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