Assay for thyroid growth stimulating immunoglobulins: 
stimulation of $[^3$H]$thymidine incorporation into 
isolated thyroid follicles by TSH, EGF, and immunoglobulins 
from goitrous patients in an iodine-deficient region

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Abstract. Estimations were carried out of $[^3$H]$thymidine incorporation during culture of isolated porcine 
thyroid follicles in the presence of standard TSH or 
epidermal growth factor (EGF) both alone and together 
with anti-TSH or anti-EGF serum, and of immunoglobulins fractionated from the sera of patients from our 
endemic goitre area with iodine deficiency. A significant 
stimulation of thymidine incorporation was 
exerted by TSH when thyroglobulin was present in the 
culture medium, and also by EGF. TSH stimulation was 
abolished by anti-TSH serum, in contrast to the effect 
of EGF. Anti-EGF serum, on the other hand, blocked 
the effect of EGF, but not that of TSH. The sera of 20 
out of 72 patients (27%) with euthyroid goitre (not 
operated on) and of ten out of 26 patients (38%) with recurrent euthyroid goitre contained immunoglobulins 
stimulating thyroid growth (TGI). There was no corre-
lation of TGI with other thyroid antibodies. TGI have 
to be regarded as a pathogenetic factor of euthyroid 
goitre also in patients from an endemic region especially 
in recurrent cases.

$[^3$H]$thymidine incorporation during culture of 
thyroid follicles isolated from porcine glands 
(Schatz et al. 1983, 1984) can be employed for 
estimating thyroid growth stimulating immunoglo-
bulins (TGI) (Drexhage et al. 1980; Chiovato et al. 1983; Valente et al. 1983; McMullan & Smyth 1984). With thyroid follicles isolated by a 
modified technique and more pure than those 
used for our first experiments, the stimulatory 
action of TSH was found less pronounced or 
sometimes even lacking. Therefore, it should be 
tested whether addition of thyroglobulin to the 
culture medium (thyroglobulin from disrupted 
follies was present to some extent in our first 
series using more impure follicles) may increase 
the responsiveness of the follicles against TSH. 
Furthermore, the effect on $[^3$H]$thymidine incorpor-
ation of TSH and epidermal growth factor 
(EGF) (Eggo et al. 1983; Westermark et al. 1985; 
Gärtner et al. 1985) should be tested both without 
and with TSH serum or anti-EGF serum present in the medium. Having established such a 
test system, TGI was assayed in sera from goitrous 
patients for evaluating the incidence and clinical 
significance of TGI in our iodine-deficient endem-
ic goitre region (cf Rotella et al. 1984).

Material and Methods

Thyroid follicles were isolated from porcine glands as 
described elsewhere (Schatz et al. 1984; cf Herzog & 
Miller 1979) using a modified technique: For mecha-
nical disruption, Pature pipettes with a minimal diameter 
of 600 (instead of 200) µm were used (Herzog 1983), 
and washing was performed with 3 × 300 ml of medium 
(instead of 2 × 50 ml). This technique resulted in much 
cleaner and better preserved follicles.

The follicles cells were estimated in a Neubauer 
aemocytometer chamber and 20 000 thyroid cells per 
well were cultivated in 180 µl of minimal essential 
medium (MEM (Earle), Boehringer/Mannheim) con-
aining 10% foetal calf serum (Gibco) together with 1 mg of thyroglobulin per ml (porcine, Type II, Sigma) in the wells of an agar-gel coated microtitre plate for 24 h. Afterwards, [3H]thymidine (0.4 µCi, Amersham, 5 mmol) was added together with either standard TSH (0.01, 0.1, 1, 10 and 100 mU/ml, from bovine pituitary, Sigma) or epidermal growth factor (EGF, from mouse submaxillary glands, Sigma, 10⁻⁹ to 10⁻⁶ mol) both alone or together with rabbit anti-TSH serum (final concentration 1:200, obtained from Paesel, Frankfurt/M), or with rabbit anti-EGF serum (final concentration 1:100, 1:200 and 1:20 000, obtained from Immuno Biological Laboratories GmbH, Hamburg). For estimating TG1, ammonium sulphate-precipitated and dialysed immunoglobulins from serum of patients (and normal controls) were added yielding final concentrations of 0.01, 0.1, 1, and sometimes also 10 mg per ml. After another 48 h of cultivation, the cells were harvested with a cell harvester (Titertek, Flow Laboratories) and incubated Ci/for 24 h with a tissue solubilizer (Soluene 350, Packard). Each concentration of TSH and EGF was tested in all wells of one row of the microtitre plate, i.e. 8- or 12-fold.

For clinical studies, the normal range (mean ± sd) was established from immunoglobulins from healthy controls. Mean [3H]thymidine incorporation in the presence of immunoglobulins from controls for all experiments was 93% (range 65–117%) of the ‘blank’ value found with medium alone. This value for the normal immunoglobulins in each individual experiment was taken as 100%. Each serum sample was tested 6 times, and the points on the figures represent the mean values for each of 6 determinations.

In the sera from all the patients with euthyroid goitre and, for comparison, also with hyperthyroid goitre, anti-microsomal and anti-thyroglobulin antibodies (MAb, TgAb) were estimated by the haemagglutination technique (Thymune M, Thymune T, Wellcome, TgAb also by radioimmunoassay (PEG-method, Serono). All sera were tested for TSH receptor antibodies (TBIAb) using the TRAK® assay (Henning). Goitre size was classified according to WHO. Statistical evaluations were carried out using Student’s t-test.

Results

In our test system, using 10% foetal calf serum, the ‘basal’ incorporation of tritiated thymidine was high and reached values between 10 000 and 15 000 cpm. TSH responsiveness of the – highly purified – isolated thyroid follicles was significantly increased in the presence of thyroglobulin up to TSH concentrations of 1 mU/ml (Fig. 1). The typical bell-shaped pattern was observed for the dose-response curve of TSH (Figs. 1 and 2): at the high concentration, 100 mU/ml, a decrease even below the zero value was found. Epidermal growth factor too proved to be a potent stimulator of thymidine incorporation (Fig. 2). Anti-TSH serum abolished the stimulatory effect of TSH but not that of EGF (Fig. 3). On the other hand, in the presence of anti-EGF serum (Fig. 4), the ‘basal’ thymidine incorporation was somewhat, though not significantly depressed. The stimulatory action of EGF on [3H]thymidine incorporation was almost blocked by anti-EGF serum at final concentrations of 1:100 and 1:200 (in contrast to 1:20 000): No significant augmentation of the thymidine incorporation was observed any more. TSH, however, still provoked a significant increase in thymidine incorporation also in the presence of anti-EGF serum at all concentrations tested.

Twenty out of 72 patients (27%) with euthyroid
goitre (diffuse or nodular, without surgery) were TGI-positive (Fig. 5). In patients with recurrent goitre after surgery, TGI were found in 10 out of 26 patients (38%). TGI were also detected in sera of untreated and in some of the treated patients with hyperthyroid goitre, mainly in those positive for TBIAb and/or with endocrine ophthalmopathy (E.O.). Patients with diffuse and with nodular euthyroid goitre were equally TGI-positive (27% and 29%, respectively). No significant increase in TGI was observed with increasing goitre size (Fig. 6). The immunoglobulins were tested at various protein concentrations. Already at low concentrations of immunoglobulins (0.01 and 0.1 mg/ml, indicating a high antibody 'titre') TGI-positivity was quite often detected in the sera from patients with recurrent goitre (in 4 and 3 patients, respectively, out of the 10 TGI-positive patients).

Table 1 shows that, out of 80 patients with euthyroid goitre, 59 were negative for TgAb, MAb, and TBIAb, although 21 of these 59 patients were 'TGI-positive' (positive at least at one of the immunoglobulin concentrations tested). Among the patients exhibiting MAb and/or TgAb, only a few were also TGI-positive.

From Table 2 it can be seen that altogether, TGI were not clearly related to the result of the TRH test. However, in patients without thyroxine treatment, TGI-positivity was found in five patients with a positive and in no patient with a negative TRH test.

Discussion

The first part of the study confirmed our previous results (Schatz et al. 1983) which had shown that TSH can promote the growth of thyroid follicles cells isolated from porcine glands. Using
Fig. 3.

\[^{3}H\]thymidine incorporation during 48 h into isolated porcine thyroid follicles in the presence of TSH or EGF alone (●●●) or together with anti-TSH serum (○○○).

Fig. 4.

\[^{3}H\]thymidine incorporation during 48 h into isolated porcine thyroid follicles in the presence of TSH or EGF alone (●●●) or together with anti-EGF serum (final concentration 1:200, ○○○).
highly purified follicles prepared according to Herzog (1983), however, addition of thyroglobulin appears to facilitate cellular events resulting in increased thymidine incorporation. A bell-shaped dose-response curve for TSH as in this study has been found also in other test systems (van der Gaag & Drexhage 1984; McMullan & Smyth 1984). In our experiments thymidine incorporation at the high TSH concentration of 100 mU/ml became even depressed below the (relatively high) ‘blank’ value (without TSH, in medium containing 10% foetal calf serum). Other authors have also obtained an inhibition of thymidine incorporation below the zero value by TSH (Gärtner et al. 1985; Watanabe et al. 1985), however, without any significant increase at lower TSH concentrations. These differences may be due to the experimental conditions, e.g. addition of thyroglobulin and/or differences in the content of foetal calf serum in the culture media.

Since the effect of EGF was blocked by anti-

EGF serum, but not the effect of TSH, the growth promoting action of TSH does not necessitate the involvement of (locally occurring) EGF. EGF, however, is certainly a potent stimulator of thyroid growth as demonstrated also in our follicle preparation.

In the second part of the study our previous findings were confirmed concerning the occurrence of TGI also in goitrous patients from an endemic goitre region with iodine deficiency. The incidence in our region is, however, lower than in other areas with higher nutritional iodine supply (Drexhage et al. 1980; Chiovato et al. 1983; Valente et al. 1983; McMullan & Smyth 1984; van der Gaag et al. 1985). In patients with recurrent goitre, the incidence (Fig. 5) and also the concentration of TGI appear to be relatively high. What therapeutic consequences should be drawn if a goitrous patient exhibits a high concentration of TGI is still under discussion (Doniach 1984).

No major difference was found in TGI between

![Graph: Thyroid Growth Stimulating Immunoglobulins in Goitrous Patients](https://via.placeholder.com/150)

**Fig. 5.**

Thyroid growth stimulating immunoglobulins (TGI) in sera of goitrous patients from our iodine-deficient endemic goitre region.
Fig. 6.
Data of Fig. 5 grouped according to goitre size (WHO I–III).

Table 1.
TGI-positivity in 80 patients with euthyroid goitre grouped according to the absence or presence of other thyroid antibodies (see Material and Methods).

<table>
<thead>
<tr>
<th></th>
<th>MAb, TgAb and TBIAb (negative)</th>
<th>MAb (positive)</th>
<th>TgAb (positive)</th>
<th>MAb and TgAb (positive)</th>
<th>TBIAb (positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>59</td>
<td>4</td>
<td>9</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>TGI (positive)</td>
<td>21</td>
<td>1</td>
<td>2</td>
<td>1</td>
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</table>
patients with diffuse and with nodular goitre (cf. van der Gaag et al. 1985) which would have corroborated the hypothesis put forward by Studer & Ramelli (1982). No clear-cut relationship could be demonstrated between TGI and goitre size (Fig. 6). It should be considered, however, that our study was a cross-sectional study so that the patients' immunoglobulins have been tested at different phases of goitre formation, i.e. in active and also in inactive periods.

From Table 1 it can be seen that TGI represent a distinct class of thyroid antibodies. In euthyroid goitre, TGI have little relation to other thyroid antibodies including TB1AAb. This has been interpreted by others to mean that, at least in euthyroid goitre, TGI do not represent an antibody against the TSH receptor. However, it might well be that this antibody recognizes other structures of the TSH receptor than those responsible for the TSH binding. TGI did not show any clear-cut relation to the function of the pituitary-thyroid axis either (Table 2). The exact pathogenetic role and the clinical significance of thyroid growth stimulating immunoglobulins have yet to be established.

References


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