EDITORIAL
Do Thyroid Growth-Promoting Immunoglobulins Exist?*

Thyroid stimulating antibody is now accepted as the cause of hyperthyroidism in Graves' disease; in at least two studies its ability to stimulate cAMP accumulation in FRTL5 cells in vitro correlated with its stimulation of cell growth (1, 2), but not with goiter size (1). Less well established, and still controversial, is the existence of an immunoglobulin G (IgG) specific for thyroid cell growth (TGI) occurring not only in goitrous Graves' disease but also in patients with goitrous Hashimoto's thyroiditis, simple and endemic goiter, and even toxic adenoma. Largely through the efforts of Drexhage and his colleagues (3) TGI is seen as not acting through adenylate cyclase and specifically not binding to the TSH receptor. Involvement of an immunoglobulin in pathogenesis implies an autoimmune disease and, a priori, one might doubt an autoimmune origin for conditions that are not associated with other autoimmune stigmata. However, the data are accumulating and have to be examined objectively.

The topic of measurement of thyroid cell growth was reviewed in careful detail by Dumont et al. (4); they raised several important criticisms of current methodology. In fact, the majority of studies of growth of thyroid cells, i.e. considering a wide variety of experiments that do not necessarily relate to TGI, predominantly use the cell's handling of 3H-thymidine ([3H]Tdr) as an index of growth. Unfortunately, use of [3H]Tdr does not necessarily reflect true growth. There is no excuse for accepting the simple uptake of [3H]Tdr, measured as 3H remaining after cells are washed, as a criterion of growth. Indeed even incorporation of the precursor into acid-precipitable (presumptively DNA) material is too readily misinterpreted as reflecting growth. In addition to the pitfalls of this method described by Dumont et al. (4) there is the problem of DNA repair occurring separate from mitosis. In this circumstance, radioautographic evidence of [3H]Tdr incorporation into nuclear DNA may be obtained despite clear evidence that there is no cell division. It would, therefore, seem reasonable to have incorporation of [3H]Tdr (into DNA) used as a screening index of growth stimulation but only with concomitant measurement of DNA. This is the least demanding procedure and is devoid of observer bias. Much more demanding, but irrefutable as reflection of replication, is a directly monitored increment in cell number or metaphase index assay (2).

Over the last decade Drexhage and colleagues (3) have made major use of a cytochemical assay for growth. This involves either the Feulgen reaction to identify cells in S phase, or assessment of glucose-6-phosphate dehydrogenase on the assumption that this enzyme is rate-limiting in cell division. Regarding the former technique, it is a time-honored procedure for quantification of cellular DNA and, as used (3), the proportion of cells having more than 1.4 times the normal DNA content is assessed; these cells are assumed to be in, or to have completed, S-phase. Technical pitfalls exist for this technique (4) but, as applied in the cytochemical TGI assay, there is a basic problem. The assay depends upon guinea pig thyroid segments reacting to TGI (or TSH) in 3-5 h and the response was shown to plateau thereafter (3). However, mammalian cells commonly take 8-12 h to enter S phase that then reaches a peak between 24 and 48 h. The data reported with the cytochemical assay clearly do not follow this pattern and one must infer a surprising uniformity of responsive cells on the verge of entering S phase.

TGI assays based upon the assessment of glucose-6-phosphate dehydrogenase activity (3) also invoke certain untested assumptions (4). Perhaps the most troublesome is the implication that an increase in the enzyme activity is an obligatory step preceding mitosis and therefore quantitatively reflects incipient cell division. Considering the multiple biosynthetic reactions that involve the hexose shunt, for which glucose-6-phosphate dehydrogenase is the enzyme first involved, this is a precarious assump-
tion that merits greater documentation than it currently has.

In view of the criticisms of cytochemical bioassay, an alternate method of assessment of TGI is now proposed by Wilders-Truschnig et al. (5) in the current issue of The Journal of Clinical Endocrinology and Metabolism, viz, one based upon a metaphase index in FRTL5 cells. This entails the culture of FRTL5 cells in medium containing 5% calf serum, five hormones (including insulin at 10 μg/ml) and 50 μU TSH/ml which induces metaphase in 2–3% of the cells. The inclusion of TSH in the system is thought to sensitize it to further stimulation and a TGI-positive preparation is therefore one that augments the TSH effect. By this technique, data similar to those obtained by cytochemical assays are presented. Yet again, however, there are matters for concern; specifically, what growth-promoting pathway is involved? For stimulation of growth in FRTL5 cells, TSH requires a permissive effect that may be provided by serum or by insulin/insulin-like growth factor-I (IGF-I) or insulin-like growth factor-II [IGF-II(6)]. Such a permissive effect being available, TSH is the most potent mitogen of FRTL5 cells. With 5% serum, not only is there no potentiation by insulin or IGF-I, but stimulation of [3H] Tdr incorporation and total DNA is near maximum with 50 μU TSH/ml medium (7). The corollary of these considerations is that the combined effects of TSH and growth factors must be studied in a very low concentration of serum, or in its total absence, in which situations they are most prominent.

As noted above, we have found 50–100 μU/ml to exert a maximal effect on growth (as indexed by an increase in DNA) of FRTL5 cells (7). Therefore, another surprising finding is the linearity of the TSH dose-response relationship in the metaphase index assay, viz, from 10 to 10,000 μU/ml. With the identical technique, Ealey et al. (2) observed a bell-shaped curve for this range of concentration of TSH. However, using IgG, Wilders-Truschnig et al. (5) consistently obtained, in combination with 50 μU TSH/ml, a bell-shaped pattern of response over a mere 5-fold range of IgG concentration. These differing patterns merit discussion. Further, it is noteworthy that the TGI-positive IgG were effective only as potentiators of TSH and, alone, even in high concentration, did not increase the metaphase index. It may be relevant to point out that concentrations of forskolin and IBMX, that were ineffective on their own, increased the TSH-stimulated metaphase index, but at higher concentrations they were stimulatory in the absence of TSH (8).

It would appear, therefore, that if TGI is being measured in this system it must be acting via a pathway that indeed is not involving cAMP, although sensitized by TSH, and is not that used by insulin/IGF-I. Since the EGF pathway (that is not active in FRTL5 cells and anti-epidermal growth factor serum was shown not to influence TGI) is not a candidate, one is left with the intriguing uncertainty of what mechanism underlies the actions of TGI.

Proof that an IgG is the active molecule for these effects is claimed by use of anti-IgG and anti-TSH; the former obviated growth stimulation and the latter had no effect. However, a molecule adsorbed or bound to IgG is liable to be precipitated by anti-IgG, together with the IgG itself. Consequently if a growth factor were bound to IgG it could be coprecipitated. Thus it may be that, in these experiments, IgG is not the bioactive molecule and a contaminating growth factor is exerting an effect while bound to IgG.

The data provided by Wilders-Truschnig et al. (5) are provocative and deserve attention. However, the fact that they confirm results obtained by the cytochemical bioassay is disturbing, considering the concerns about that procedure summarized above. It is to be hoped that multiple assays, using unequivocal measures of cell proliferation, will be applied to this important topic to confirm or refute the existence of TGI in conditions, such as endemic and sporadic non toxic goiter, not otherwise viewed as autoimmune in origin. It would be most propitious if this group made available to at least a few other laboratories appropriate quantities of the potent sera that they have used for this report. That way, vital independent confirmation and extension of the findings would rapidly be attempted. In addition, one must recognize that none of the TGI experience has been obtained with human thyroid cells or tissue. Consequently, while effects on FRTL5 cells probably represent what may be expected to some extent with human material [and Dumont et al. (4) showed that human thyroid cells in primary culture respond similarly, in terms of growth, to TSH and insulin], it should be kept in mind that these cells are neither human nor truly normal. Unfortunately, it is unlikely that confirmatory experiments using normal human thyroid cells will be carried out in the near future because of the logistic difficulty of obtaining sufficient material.

The present data clearly suggest that in some patients there exists an IgG that stimulates growth of FRTL5 cells, but only in the presence of TSH. The mechanism of effect is probably not through stimulation of adenylate cyclase. The interpretation must be that there is either a contaminating growth factor or the IgG contains an antibody acting as agonist on a receptor for such a factor that presumably is not insulin/IGF-I and is not present in calf serum. Confirmation of these findings and then further elucidation of the mechanisms involved, are developments that will be awaited with great interest.
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References  