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THYROID AUTOIMMUNITY IN ENDEMIC GOITRE CAUSED BY EXCESSIVE IODINE INTAKE*

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SUMMARY

The pathophysiology of endemic goitre caused by excessive iodine intake is not well defined. By interacting with the immune system, iodine excess may trigger the development of autoimmune thyroid disease such as lymphocytic Hashimoto's thyroiditis (LT). In an attempt to examine this further, we compared the presence of thyroid autoantibodies in 29 goitrous children, from an iodine excess area, and in 26 healthy children, from an iodine sufficient area, of north central China. Serum was tested for antimicrosomal (MAb), anti-thyroglobulin (TgAb), second colloid antigen antibodies (CA2-Ab) and TSH binding inhibitory immunoglobulins (TBII). Affinity chromatographically purified IgG was tested for thyroid growth-stimulating activity (TGI) by two different methods: a sensitive cytochemical bioassay (CBA) using guinea-pig thyroid explants and a mitotic arrest assay (MAA) employing a continuous rat thyroid cell line (FRTL-5). We found no increased prevalence of LT in patients with endemic iodine goitre. The levels of MAb, TgAb and CA2-Ab did not differ significantly between the two groups of children. Further, TBII were not present in either group. Thyroid growth-stimulating immunoglobulins (TGI) were the major autoantibodies found in children with goitres caused by iodine excess. In the CBA, 12 of 20 (60%) goitrous children and 0 of 12 (0% $P<0{\cdot}05$) healthy children were positive for TGI. Similar results were found in the MAA, and a good correlation between results of the CBA and MAA was found ($P=0{\cdot}003$). Maximal TGI activity in dose–response CBA showed a good relation with clinical goitre size ($r=0{\cdot}63$; $P<0{\cdot}05$) indicating a possible pathophysiological role for these antibodies. We conclude that endemic iodine goitre is not

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associated with Hashimoto's lymphocytic thyroiditis. Nevertheless, autoimmune growth factors such as TGI may play a primary role in the pathogenesis of thyroid growth in this condition.

Intake of excessive iodine, either in food, water or drugs, may result in disturbances of thyroid function and growth (Roti 1985; Monteiro *et al.*, 1986; Martino *et al.*, 1987). Sporadic iodide goitre is rare, invariably associated with thyroid hormone deficiency, and develops as a consequence of high levels of serum thyrotrophin (TSH) (Braverman, 1985). Hypothyroidism results from the thyroid gland's inability to escape from the inhibitory effect of iodide on thyroid hormone release and production (Wolff, 1964, 1969). This failure to adapt to an iodine load has been described in immature infants, patients with underlying thyroid disease, chronic respiratory illness, and those ingesting co-incident goitrogens (Braverman, 1985).

We have previously described an endemic iodine goitre area in north central China, due to iodine-rich water, where goitre affects up to 65% of schoolchildren (Li *et al.*, 1987). The pathophysiology of this condition is poorly understood. In contrast to sporadic cases of iodide goitre, clinical and biochemical hypothyroidism is unusual in the endemic type (Suzuki *et al.*, 1965). We found, in our previous study, that tests of thyroid function were normal in two-thirds of chidren. In the remaining one-third, mild elevations of serum TSH with normal serum thyroxine (T4) levels (subclinical hypothyroidism) were present (Li *et al.*, 1987). Thyroid glands were firm, diffusely enlarged, with an uneven surface. Occasionally, the larger glands were distinctly multinodular, similar to Hashimoto's lymphocytic thyroiditis (LT).

Epidemiological and experimental studies have suggested a link between the level of iodine intake and the development of autoimmune thyroid disease (McGregor *et al.*, 1985; Phillips *et al.*, 1988). In this report, we examined the prevalence of thyroid autoantibodies in subjects with endemic iodine goitre to ascertain the possible pathogenetic role of thyroid autoimmunity in this condition. Our results indicate that antibodies which promote thyroid growth (TGI) are found in a high percentage of patients with endemic iodine goitre. The prevalence of Hashimoto's thyroiditis, however, was not increased in the iodine excess population.

BACKGROUND AND PATIENTS

The epidemiological and biochemical findings have previously been described (Li *et al.*, 1987) and are summarized in Table 1. Briefly, Gaojiabu village (iodine concentration 462·5 μg/l of drinking water) is 600 km from the coast in Shanxi Province, Central China. From 316 pupils at the local primary school, 120 children (ages 7 to 15 years) were examined. Huanglo village (iodine concentration 54 μg/l of drinking water) served as a control where 51 schoolchildren (ages 7–15 years) were examined. Thyroid status was assessed and goitre size was graded using WHO criteria. Urinary iodine and creatinine concentrations were measured using the Technicon Auto-analyzer II (Garry *et al.*, 1973). Serum was taken randomly from 29 and 26 subjects from Gaojiabu and Huanglo, respectively. Serum TSH concentrations were assayed by a sensitive immunoradiometric assay (Sucrosep TSH IRMA; Boots-Celltech Diagnostics UK). Serum total triiodothyronine (T3), thyroxine (T4), and free T4 concentrations (FT4) were measured with commercial radioimmunoassay (RIA) kits (Baxter Travenol Diagnostics, Cambridge, MA.).

Table 1. Epidemiological and biochemical features of the iodine excess and iodine sufficient villages

	Iodine excess (Gaojiabu)	Iodine sufficient (Huanglo)
Clinical thyroid status	Euthyroid	Euthyroid
Goitre prevalence	65%	15%
Goitre-Grade 2	15%	0%
Iodine/drinking water	463 μg/l	54 μg/l
Urinary iodine/creatinine	1236·5 ± 1·5 μg/g	428·4 ± 3·4 μg/g
Free thyroxine (FT4) (NR 11·1–25·0)	19·1 ± 3·4 pmol/l	16·2 ± 3·0 pmol/l*
Total triiodothyronine (T3) (NR 1·2–2·8)	1·6 ± 0·3 nmol/l	1·9 ± 0·2 nmol/l†
Thyrotropin (TSH) (NR 0·1–6·0)	5·2 ± 2·1 mIU/l	3·9 ± 1·0 mIU/l‡

Results are mean ± SD.
*$P < 0.05$; †$P < 0.001$; ‡$P > 0.05$.

From Gaojiabu, serum TSH levels (normal reference range, 0·1–6·0 mU/l) were elevated in 11 of 29 (38%) individuals, but only one case showed serum TSH levels greater than 10 mIU/ml. In all cases except the latter, serum T4 levels were in the normal range (70–170 nmol/l).

METHODS

Thyroid cytoplasmic and colloid antibodies

Antithyroglobulin (TgAb) and antimicrosomal (MAb) antibodies were determined by commercial haemagglutination assays (Wellcome, Beckenham, UK). Second colloid antigen-antibodies (CA2-Ab) were determined by indirect immunofluorescence on frozen human thyroid tissue obtained from surgery as previously described (Balfour *et al.*, 1964). Briefly, cryostat sections (5μm) of these thyroids were fixed in 100% methanol (60 min at 25°C) followed by a post-fixation of 10 s in 70% methanol. Patient ($n = 28$) and control ($n = 17$) sera were tested in dilutions of 1 : 10 to 1 : 80 in phosphate buffered saline (PBS), pH 7·4. Sections were incubated with diluted sera, washed twice in PBS, incubated with fluorescein-conjugated calf anti-serum to human total immunoglobulins (Kallestad, Austin, Texas; dilution 1 : 120), and thereafter two washes in PBS. Sections were mounted in Aquamount (BDH Chemicals Ltd, Poole, England) and kept in the dark at 4°C until evaluation.

Thyroid binding inhibitory immunoglobulins (TBII)

Serum was tested for TSH receptor autoantibodies using a commercial radioreceptor assay (Trak Assay, Henning, Berlin). The normal reference range for this test in our laboratory is a value less than 10%.

Thyroid growth-stimulating immunoglobulins (TGI)

Preparation of immunoglobulin

Purified immunoglobulin G (IgG) was prepared from patient serum by affinity chromatography on Protein-A sepharose CL-4B (Pharmacia, Uppsala, Sweden). Glycine hydrochloride (HCl) buffer (pH = 2·8) was used to elute bound IgG from the column and its appearance in the elute was monitored by a u.v. path spectrophotometer. The resultant preparation was then dialysed overnight against phosphate buffered saline (pH = 7·4) and the IgG content of the eluate was determined spectrophotometrically at $\lambda = 280$ nm. The preparations were stored at −20°C. Control IgG preparations were prepared in an identical manner.

Cytochemical bioassay

Thyroid growth-stimulating immunoglobulins (TGI) were determined in duplicate by measuring DNA synthesis in guinea-pig thyroid explants using Feulgen cytophotometry as previously described in detail (Drexhage *et al.*, 1980; Van der Gaag *et al.*, 1985). The amount of stain in 50–100 individual thyroid follicle-cell nuclei from three to six separate sections was quantified in a randomized way with a Vickers M85 microdensitometer ($\lambda = 550$ nm, ×100 oil-immersion objective, 0·2 μm scanning spot). The relative absorption values (relative DNA content values per nucleus) are plotted as population histograms, from which the diploid (2c) content can be defined. From previous studies (Drexhage *et al.*, 1980), cells with a content of more than 2·8 c were taken as being in, or having completed the S-phase of the cell cycle. All experiments were carried out on coded samples and results were expressed as the percentage of cells in S-phase with a normal reference range below 5%.

Samples were tested at a single concentration of IgG (50 μgIgG/ml) in 20 patients from the iodine excess village (Gaojiabu) and 12 from the control village (Huanglo). In addition, a subset of samples (12 from Gaojiabu; five from Huanglo) were tested by titration at concentrations of IgG 0, 15, 30, 50 and 100 μgIgG/ml of culture fluid. In all assays, a guinea-pig thyroid segment was maintained without IgG (the control culture), and in another thyroid segment, bovine TSH (1 μU/ml) was added as a positive control. Positivity at 30–50 μgIgG/ml in titration assays was defined as strongly positive for TGI.

FRTL-5 mitotic arrest assay (MAA)

FRTL-5 cells were cultured under standard conditions; Ham's F-12 medium with Coon's modification, containing 5% calf serum and six-hormone (6H) mixture including bovine TSH (Sigma Chemicals) at a concentration of 1 mU/ml (Ambesi-Impiombato *et al.*, 1980; Bidey *et al.*, 1988). Cells were seeded into 48-well plates (4×10^4 cells/ml) and cultured for 4 days in 6H. After this period, cells were washed twice with Hanks' Balanced Salt Solution (Gibco) and changed to the same medium without TSH (5H) for a further 4 days. To test for TGI activity, cells were co-stimulated in 5H plus a suboptimal dose of TSH (50 μU/ml; 5H+) as proposed by Wilders and co-workers (Drexhage *et al.*, 1988). To this mixture, test or control IgG was added in a concentration of 1000 μg/ml and cells incubated at 37°C and 5% CO_2 for 47 h. Colcemid (Sigma Chemicals) was added during the last 6 h of culture, cells were air-dried and stained with May–Grünwald–Giemsa. The number of mitotic figures (per 500 cells) was counted directly in the wells, using an

Table 2. Thyroid autoantibody results

	MAb	TgAb	CA2-Ab	TBII	TGI CBA	TGI MAA
Iodine excess	6% (2 of 29)	6% (2 of 29)	29% (8 of 28)	0% (0 of 29)	60% (12 of 20)*	68% (13 of 19)*
Iodine sufficient	4% (1 of 26)	4% (1 of 26)	18% (3 of 17)	0% (0 of 17)	0% (0 of 12)	0% (0 of 12)†

* $P < 0{\cdot}05$ (chi-squared test).

† Includes six controls from an iodine sufficient Dutch population.

MAb, anti-microsomal antibody; TgAb, antithyroglobulin antibody; CA2-Ab, second colloid antigen antibody; TBII, Thyroid binding inhibitory immunoglobulins; TGI, thyroid growth stimulating immunoglobulins; CBA, cytochemical bioassay; MAA, mitotic arrest assay.

inverted microscope as described by Ealey *et al.* (1988), and a mitotic index calculated. Triplicate wells were measured for each test stimulator. Growth index was expressed as:

$$\frac{\text{Mitotic index (IgG plus TSH [50 } \mu\text{U/ml])}}{\text{Mitotic index (TSH [50}\mu\text{U/ml])} \times 100\%$$

Therefore by definition cells grown in 5H + (TSH[50 μU/ml]) had a growth index of 100%. TGI positivity was defined as a growth index two standard deviations above growth obtained with 5H + alone.

Statistics

Statistical evaluation utilized Chi-squared analysis with a continuity correction factor and linear regression analysis. Results are presented as mean and standard deviation (SD). The study was approved by the Ethics Committee of Westmead Hospital.

RESULTS

Prevalence of antibodies directed against cytoplasmic and colloidal antigens

Thyroid autoantibody data in the iodine excess group are presented in Table 2. Only one patient from the iodine excess population demonstrated the biochemical and autoantibody features of lymphocytic Hashimoto's thyroiditis. This patient had a large goitre (grade 2), was hypothyroid (TSH = 60 mIU/l) and hypothyroxinaemic (T4 = 33 nmol/l). MAb (1 : 1600), CA2-Ab (1 : 80), and TGI (50 μgIgG/ml) were markedly positive.

In the remainder, the prevalence of MAb, TgAb, and CA2-Ab was similar in iodine excess and control groups. One of 29 (3%) children from Gaojiabu demonstrated positive MAb and TgAb titres compared with one of 26 (4%) children from Huanglo ($P > 0{\cdot}05$). The frequency of CA2-Ab present in the iodine excess population, eight of 28 (29%), was not significantly different from control areas, three of 17 (18%; $P > 0{\cdot}05$). CA2-Ab titres ranged between 1 : 10 and 1 : 80.

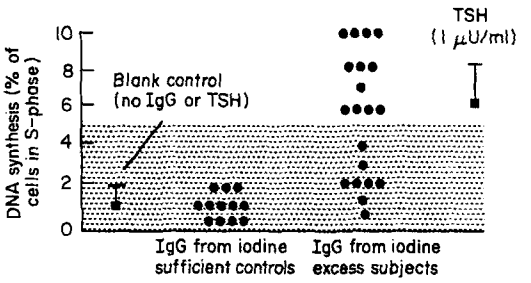

Fig. 1. Stimulation of thyroid growth *in vitro* induced by IgG preparations (tested at a single concentration of 50 μg IgG/ml) of patients with iodine excess goitre (Gaojiabu) compared with iodine sufficient controls (Huanglo). The shaded area represents normal range in the cytobiochemical assay. Each *dot* represents the mean of two duplicate experiments. In each assay, one guinea-pig thyroid segment was kept without IgG or hormone added (negative controls) and one segment served as a positive control (1μU/ml bovine TSH).

Antibodies directed against the TSH receptor

IgGs which inhibit the binding of ^{125}I-labelled TSH to its receptor were not detected in either iodine excess goitrous patients or in iodine sufficient controls (Table 2). A positive result was regarded as a value of greater than 10%.

Thyroid growth-promoting antibodies (TGI)

Cytochemical bioassay

These thyroid autoantibodies were the predominant autoantibody detected in the iodine excess population. When tested at a single concentration of IgG (50 μgIgG/ml), 12 of 20 (60%) patients from the iodine excess village were positive for TGI (Fig. 1). This incidence of TGI was significantly different from iodine sufficient villagers, none of 12 (0%) [$\chi^2 = 6{\cdot}3$; $P = 0{\cdot}01$].

To determine the potency of circulating TGI, titration assays were performed on purified IgG of 17 subjects (12 iodine excess, five control). Biphasic dose–response curves were obtained, a characteristic finding of cytochemical bioassays (CBA) (Drexhage *et al.*, 1980) (Fig. 2a, b). Maximal TGI activity in iodide-induced goitre subjects was found at concentrations of 30–50 μgIgG/ml.

When tested in titration assay, circulating TGI demonstrated a good correlation ($n = 17$; $r = 0{\cdot}63$; $P < 0{\cdot}01$) with clinical assessment of goitre size. In contrast, serum TSH concentrations did not correlate with either TGI activity ($r = < 0{\cdot}01$; $P = 0{\cdot}9$) or thyroid gland size ($r = 0{\cdot}14$; $P = 0{\cdot}3$). Furthermore, as would be expected, urinary iodine concentrations also did not correlate with goitre size ($r = 0{\cdot}03$; $P = 0{\cdot}9$) or with TGI activity ($r = 0{\cdot}3$; $P = 0{\cdot}5$).

FRTL-5 mitotic arrest assay

FRTL-5 cells grown in the absence of TSH showed little or no growth, mean mitotic index (MI), $1{\cdot}4 \pm 0{\cdot}5\%$ ($n = 15$) (Fig. 3). With the addition of submaximal concentrations of TSH (50 μU/ml) the percentage of cells in mitosis increased, $3{\cdot}7 \pm 0{\cdot}5\%$ ($n = 25$; $P < 0{\cdot}05$). The growth response to increasing doses of TSH was biphasic and reached a

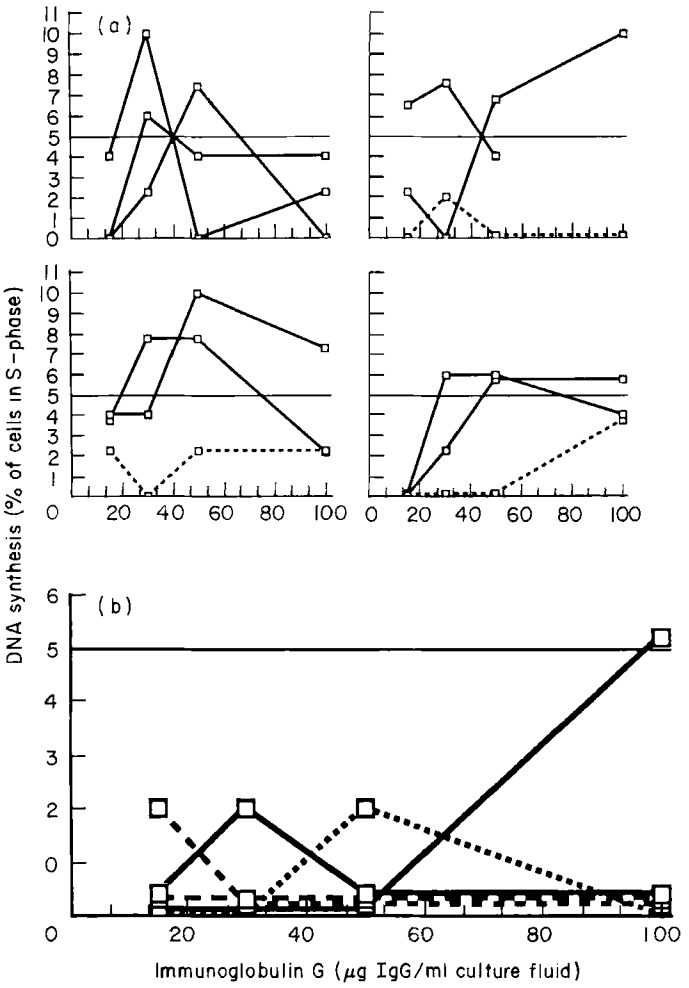

Fig. 2. Stimulation of thyroid growth tested in dose response *in vitro* at IgG concentrations of 15, 30, 50 and 100 μg IgG/ml of culture fluid in a, iodine excess ($n = 12$) and b, control ($n = 5$) subjects. DNA synthesis less than 5% represents expected normal range. Open squares with unbroken line indicate a positive TGI result ($n = 9$); open squares with broken line indicate negative thyroid growth stimulating activity (TGI). In b, one of five control subjects showed minimal TGI activity. A characteristic feature of cytobiochemical assays is a biphasic (bell-shaped) dose-response curve.

maximum at a concentration of 1 mU/ml (6H) where the mean MI was $6{\cdot}5 \pm 1{\cdot}5\%$ ($n = 15$). Control IgG preparations from euthyroid iodine sufficient patients from Holland ($n = 6$) and China (Huanglo) ($n = 6$), added to cells with 5H+, did not promote additional thyroid cell growth (Fig. 3). In contrast, IgG from patients with endemic iodine goitre showed no TGI activity when cultured in the absence of TSH; but in the presence of 5H+ potentiated the mitogenic effects of TSH (Fig. 3). TGI activity was detected in 13 of 19 (68%) IXS patients tested and in 0 of 12 controls ($P < 0{\cdot}05$).

Results of FRTL-5 MAA were compared with results in the CBA (Table 3). Compared to the latter, the sensitivity and specificity of the MAA were 89 and 79%, respectively

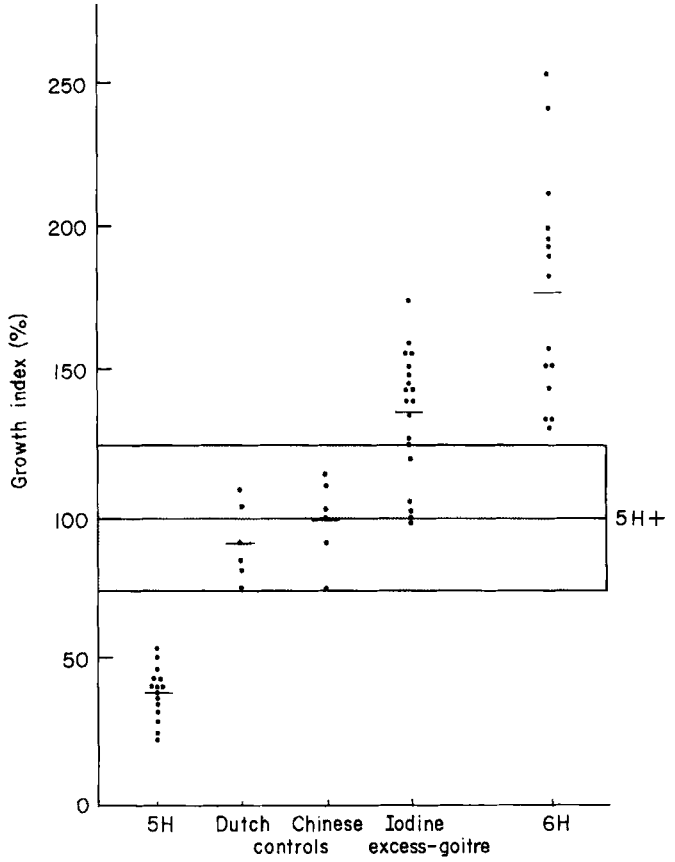

Fig. 3. Growth index of rat thyroid cells (FRTL-5) cultured in five hormone mixture (5H, without added TSH); six-hormone mixture (6H, TSH added at a concentration of 1 mU/ml); iodine sufficient control patient immunoglobulin G (IgG) plus TSH (50 μU/ml); and IgG from iodine excess goitrous patients plus TSH (50 μU/ml). Each point is the mean result of three wells. The boxed area represents the normal range (± 2 SD) for growth index in FRTL-5 cells cultured with 5H+ (TSH 50 μU/ml) without patient IgG. Samples above the boxed area were considered as TGI positive.

(Table 3). The positive predictive value (PV) and negative PV of the MAA were 67 and 94%, respectively. DNA synthesis (measured in the CBA) and mitotic index (FRTL-5 cells) showed a positive relation ($r=0{\cdot}6$; DF 26; $P=0{\cdot}001$).

DISCUSSION

In this report, we examined the relationship between autoimmune thyroid disease and endemic iodine goitre. The prevalence of seropositive Hashimoto's lymphocytic thyroiditis (Baker *et al.*, 1988) (LT) was not increased in the iodine excess study group. Nevertheless, thyroid growth-stimulating immunoglobulins (TGI) were present in 60% of goitrous children with endemic iodine goitre and were found in the absence of other TSH

Table 3. Correlation of cytochemical bioassay (CBA) and FRTL-5 mitotic arrest assay (MAA), observed frequency table

	Cytochemical bioassay		
	Positive	Negative	Total
FRTL-5 MAA*			
Positive	8	4	12
Negative	1	15	16
Total	9	19	28

Chi-squared (with continuity correction factor) 8·9; $P = 0{\cdot}003$.

* Sensitivity 89%; specificity 79% compared with CBA.

receptor autoantibodies. The high prevalence of TGI in these patients and the correlation of TGI with goitre size suggest a pathogenic in-vivo role for these antibodies.

Previous studies suggested that LT may contribute to the development of goitre in endemic iodine excess areas (Suzuki, 1980). This assumption was based largely on indirect evidence extrapolated from human and animal studies, which looked at the immunological effects of iodine supplementation on the thyroid gland in states of iodine deficiency or susceptibility to autoimmune thyroiditis. The frequency of LT in population studies has been shown to rise after iodine prophylaxis (McConahey *et al.*, 1962; Weaver *et al.*, 1969). Specifically, thyroid autoantibodies (MAb and TgAb) are detected after iodized oil injections in humans (Boukis *et al.*, 1983) and animals (Follis, 1959). In these hyperplastic thyroid glands (secondary to iodine deficiency) there is a rapid infiltration of lymphocytes and dendritic cells associated with thyroid follicular cell necrosis (Toussaint-Demylle *et al.*, 1988). In the BB/W rat (Allen *et al.*, 1987) and obese chickens (Bagchi *et al.*, 1985), both of which develop autoimmune thyroiditis spontaneously, increasing iodine in the diet accelerates the development of thyroiditis. Our study differs from these other studies since iodine deficiency was not present but rather lifelong exposure to excess iodine. The metabolic status of the thyroid gland appears to be an important determinant of its immunological response to inordinate iodine administration. In endemic iodine goitre LT is not a feature and these findings have now been confirmed in adults as well as children (Li *et al.*, 1988). Our data are supported by histology from the Hokkaido endemia which show features of 'colloid goitre' rather than autoimmune thyroiditis (Suzuki *et al.*, 1965).

The expected incidence of TGI in non-goitrous iodine sufficient populations is less than 5% (Van der Gaag *et al.*, 1985). Thyroid growth-promoting antibodies (TGI) are found in patients with Graves' disease, recurrent Hashimoto's thyroiditis and sporadic euthyroid multinodular goitre (MNG) (Drexhage *et al.*, 1980; Van der Gaag *et al.*, 1985). TGI activity in endemic iodine goitre is of a similar potency to that described in Graves' disease. Whereas in Graves' thyrotoxicosis, a good correlation between TGI and TBII can be found (Bliddal *et al.*, 1987), in our study TBII activity was absent. This finding implies that TGI associated with iodine excess recognize antigenic determinants distinct from the TSH binding site possibly directed against a putative growth receptor closely linked to the TSH receptor domain. Monoclonal antibody studies against the TSH

receptor have confirmed that thyroid follicular cell growth and function may be stimulated independently (Valente *et al.*, 1982).

There is controversy whether TGI are a separate class of thyroid autoantibody, distinct from other thyroid-stimulating autoantibodies (Zakarija *et al.*, 1988), or an assay artifact. This is partly due to the lack of a simple, reliable assay for DNA synthesis. The cytochemical bioassay (CBA) is the most sensitive and reproducible method (Drexhage & Van der Gag, 1986), but is labour intensive and available to only a few laboratories. The mitotic arrest assay (MAA) (Ealey *et al.*, 1988), modified by Wilders and co-workers (Drexhage *et al.*, 1988), is a simpler method and its results show an excellent correlation with thyroid growth in the CBA. Compared to the CBA, the most sensitive measure of TGI, the MAA, has a high sensitivity (89%) and specificity (79%). These data refute previous claims to the contrary that the cytochemical method does not measure cellular growth (Dumont *et al.*, 1987). Dumont *et al.* (1987) have also claimed that TGI activity may be related to contamination by other growth factors and hormones in immunoglobulin preparations. There is no question that ammonium sulphate precipitation, to separate IgG from serum, does result in significant residual amounts of epidermal growth factor (EGF) and other proteins. In the present study, however, we used affinity chromatography on Protein-A sepharose, specific for the Fc receptor on immunoglobulin G, which results in purified subtypes of IgG (Brown *et al.*, 1986). Thus contamination by TSH or other growth factors does not explain TGI activity in endemic iodine goitre.

The mechanism by which iodine excess stimulates the development of TGI is unclear but iodine may interact with the immune system at several different levels. Antigen-presenting cells (APC) may be dendritic cells (Kabel *et al.*, 1988) or derived from thyroid follicular cells by the inappropriate expression of class II antigens (Bottazzo, 1986). These APC process and deliver antigen to T helper cells (Bottazzo, 1986) and are an initial determinant of autoimmune disease. Metrizamide, an iodine-rich compound, induces circulating human monocytes to develop into functioning dendritic cells (personal communication, P.J. Kabel, 1988). Another iodine-rich drug, amiodarone, results in disturbances of T cell regulation (Rabinowe *et al.*, 1986). Weetman *et al.* (1983) have shown that iodide *in vitro* enhances immunoglobulin synthesis by normal human peripheral blood lymphocytes stimulated with pokeweed mitogen. Further to its effects on antigen delivery and lymphocyte function, iodine may also augment the immunogenicity of certain thyroidal antigens, notably thyroglobulin (Sundick *et al.*, 1987).

Our prior studies have shown that serum TSH levels are usually normal or mildly elevated in most patients with endemic iodine goitre (Li *et al.*, 1987). Nevertheless, it is unclear whether TSH even at low levels is a contributing factor in the development of thyroid growth in this condition. Although TSH is considered to be the major physical regulator of human thyroid follicular cell growth and differentiation *in vivo*, it is only recently that this effect has been demonstrated experimentally (Roger *et al.*, 1988). Moreover, recent studies have shown that a number of other hormones, peptide growth factors, cytokines, and autoantibodies may modulate thyroid DNA synthesis and function (Laurent *et al.*, 1987). These other hormones and growth factors may potentiate the mitogenic effects of TSH. In FRTL-5 cells, IgG from children with endemic iodine goitre synergizes with low concentrations of TSH to stimulate thyroid growth. It is tempting to speculate whether a similar mechanism is acting *in vivo*.

The role of genetic or familial factors in endemic iodine goitre is unknown. There are no family studies or histocompatibility linked antigen (HLA) associations described, but

these important determinants cannot be excluded as playing a part, especially in small, isolated communities. Another genetic determinant could be the presence of clones of thyroid follicular cells with a high intrinsic growth potential (Smeds *et al.*, 1987). When thyroid cells are exposed to a goitrogenic stimulus, such as TSH or TGI, a preferential proliferation of these thyrocytes (with high intrinsic growth potential) will result in autonomous growth. Further, Peter *et al.* (1988) have suggested that cells that initiate nodule formation in human multinodular goitres reflect the persistence of cells with foetal growth potential in the adult thyroid gland. High intrathyroidal iodine levels *in utero* or early after birth in patients with endemic iodine goitre, associated with elevated levels of TSH, could lead to a preferential selection of these rapidly proliferating cells. Alternatively, TGI found in these patients may reflect antibodies which are directed against a persistent foetal thyroidal growth receptor or antigen.

In summary, we provide evidence for a causal link between endemic iodine goitre and specific thyroid growth-stimulating immunoglobulins. Contrary to other situations of iodine excess, endemic iodine goitre is not associated with an increased incidence of classical lymphocytic thyroiditis.

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