Clin. exp. Immunol. (1992) **87**, 261–265

The role of interferon-gamma in lymphocytic thyroiditis: its functional and pathological effect on human thyrocytes in culture

A. W. C. KUNG, L. MA* & K. S. LAU *Departments of Medicine and *Pathology, University of Hong Kong, Queen Mary Hospital, Hong Kong*

(Accepted for publication 14 October 1991)

SUMMARY

Interferon-gamma (IFN-γ) has been recognized to possess diverse non-immunological effects on epithelial cells such as cellular growth and differentiation. We have previously demonstrated that IFN-γ suppressed thyroid-stimulating hormone (TSH)-stimulated thyroglobulin (TG) synthesis in human thyrocytes through inhibition of TG gene transcription. To define the pathological mechanism involved in the action of IFN-γ, we studied the ultrastructural changes of human thyrocytes cultured in monolayer. Stimulation of the thyrocytes with TSH 10 mU/ml for 2 days resulted in marked increase in TG release into the medium. This was accompanied by elongation of microvilli, increase in follicles and acinar formation, increase in secretory granules and prominence of Golgi apparatus and rough-surfaced endoplasmic reticulum. Addition of IFN-γ (500 U/ml) resulted in marked degeneration with shrinkage of the cell membrane, vacuolation of cytoplasm, swollen mitochondria and presence of lysosomal granules. Co-culturing the thyrocytes with the IFN-γ and TSH resulted in suppression of the morphological responsiveness to TSH. There was also suppression of TSH-induced TG secretion. However, at 500 U/ml IFN-γ did not cause lysis of the thyrocytes as estimated by the cellular DNA content. Furthermore, binucleated cells were frequently encountered in those wells that were treated with IFN-γ for either 2 or 5 days. The findings suggest that IFN-γ resulted in de-differentiation and degeneration of the thyrocytes, which subsequently regained the growth potential and showed attempts at regeneration. This may explain why most patients with lymphocytic thyroiditis recover from the acute injury and do not suffer from permanent hypothyroidism.

Keywords interferon-gamma human thyrocytes ultrastructural changes lymphocytic thyroiditis

INTRODUCTION

Interferon-gamma (IFN-γ) is a cytokine released by activated lymphocytes in response to viral infection or immunopathological conditions. It is now well recognized that IFN-γ possesses not only immunological action, but also diverse non-immunological effects, such as cellular growth and differentiation.

We have previously reported that IFN-γ suppressed thyroid stimulating hormone (TSH)-stimulated thyroglobulin (TG) synthesis in human thyrocytes cultured in monolayer through TG gene transcription [1]. Furthermore, TG release and secretion into the culture medium was also inhibited. These data suggest that IFN-γ released from the infiltrating lymphocytes may play an important role in causing thyroid dysfunction in patients with various forms of thyroiditis. The exact mechanism by which IFN-γ affects thyrocytes function is unclear. We have shown that IFN-γ probably acts at a site distal to cAMP action as IFN-γ does not affect TSH-stimulated cAMP production. To define the pathologic mechanism involved in the action of IFN-γ, we studied the function and morphology of human thyrocytes cultured in monolayer using electron microscopy.

Correspondence: Annie W. C. Kung, Department of Medicine, University of Hong Kong, Queen Mary Hospital, Pokfulam Road, Hong Kong.

MATERIALS AND METHODS

Culture of human thyrocytes

Normal human thyroid tissues were obtained from para-adenomatous tissues from patients undergoing thyroidectomy for benign thyroid adenoma. The tissues were trimmed and finely minced with scissors. The tissues were then digested with 1·5 mg/ml collagenase type IV (Sigma, St Louis, MO) in Ca^{2+}-, Mg^{2+}-free Earl's balanced salt solution. After digestion, the thyrocytes were adjusted to 1×10^5 cells/ml in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (FCS). The cells were allowed to grow on glass coverslips in 6-well culture plates (GIBCO) and incubated at 37°C in a humidified atmosphere at 5% CO_2 in air until confluence.

Before the experiment, the cells were washed twice with phosphate-buffered saline (PBS). The thyrocytes were then

Table 1. Cellular DNA contents of thyrocytes cultured with thyroid stimulating hormone (TSH) or IFN-γ for 2 days

	DNA (μg/well)
Control medium	3·09 ± 0·12
TSH 10 mU/ml	3·28 ± 0·46
IFN-γ 500 U/ml	3·33 ± 0·34
TSH + IFN-γ	3·03 ± 0·71

Results are mean ± s.d.

stimulated with either bovine TSH 10 mU/ml (Sigma), recombinant human IFN 500 U/ml (Boehringer, Ingelheim, Austria) or both together for 2 days or 5 days. We studied IFN-γ and TSH at such a dose as our previous report showed that a lower dose of IFN-γ did not affect thyrocyte function and TSH 10 mU/ml gave maximal functional stimulation [1].

Electron microscopy

The thyrocytes cultured on glass plates were washed with PBS and fixed with 2·5% glutaraldehyde in 0·1% cacodylate buffer, pH 7·4 for 60 min at 4°C, then washed in cacodylate buffer with 0·1 M sucrose. The monolayers were then post-fixed with 1% osmium tetroxide for 1 h at room temperature and then dehydrated with a series of graded ethanol.

For transmission electron microscopy (TEM) studies, the cells were infiltrated with epoxy resin. Ultra-thin sections were cut, stained with lead citrate and saturated uranyl acetate and examined in a JEOL JEM-100SX transmission electron microscope at 80 kV.

For scanning electron microscopy (SEM) studies, the cells were critical point-dried and coated with a gold–palladium film and were examined in a Cambridge Stereoscan 150 scanning electron microscope at 20 kV.

Estimation of cellular DNA content

The diphenylamine method was used as described by Burton [2]. Briefly, the cells were extracted with 5% tricholoracetic acid and the DNA developing solution (consisting of diphenylamine, glacial acetic acid, H_2SO_4 and acetaldehyde) was added to the supernatant and allowed to react overnight at room temperature. DNA in the samples was estimated by determination of optical density at 260 nm using calf thymus DNA (Sigma) as standard.

Determination of TG

TG released into the medium was measured by a double antibody radioimmunoassay (RIA) [3].

RESULTS

Cellular DNA estimation

When the thyrocytes were studied after the monolayers became confluent, there was no difference between the DNA contents of the cells cultured in either conditioned medium, TSH, IFN-γ alone or in combination (Table 1). Thus IFN-γ at the dose of 500 U/ml did not cause lysis of the cell. Similar findings were observed in cells cultured for either 2 or 5 days.

TG synthesis

Similar to our previous report, addition of TSH 10 mU/ml for 2 days caused a significant increase in TG release into the medium (28·7 ± 1·2 *versus* 7·7 ± 1·8 ng/ml). IFN-γ 500 U/ml did not affect the basal TG synthesis but suppressed the TSH-stimulated TG release to 22·5 ± 1·3 ng/ml ($P < 0·05$). Similar findings were observed with thyrocytes being stimulated for 5 days, except that the amount of TG released was two-fold higher (data not shown).

Scanning and transmission electron microscopy

Thyrocytes cultured in control medium were round and polygonal in shape. There were few microvilli on the cell surface after 2 days (Fig. 1a) but they increased in number when cells were cultured for 5 days. Tight junctions were seen between cells and cytoplasmic organelles were sparsely distributed in the cytosol (Fig. 1b). Addition of TSH to the culture medium caused marked increase in follicle and acinar formation. There was also an increase in the length and number of the microvilli and increased pseudopod formation on the cell surface (Fig. 1c). The cells were filled with many lipid granules and lysosomes. There was also an increased number of ribosomes, as well as prominence of Golgi apparatus, rough-surfaced endoplasmic reticulum, and mitochondria (Fig. 1d). The amount of cytoplasmic filaments however, was similar when compared with cells in resting state.

Addition of IFN-γ resulted in change in morphology of the thyrocytes. Marked degeneration with shrinkage of cell membrane was evident on SEM (Fig. 2a). There was loss of microvilli and the cell membrane appeared disrupted. The cells became flattened, spindle shaped, with increase in the nuclear/cytoplasmic ratio. The chromatins were less granulated, and binucleated cells were frequently seen (Fig. 2b). In the cytoplasm, two striking organelle changes were seen. First, there were lysosomal granules, vacuoles, residual bodies and electron-dense laminated bodies which were not present in normal cells. Second, the mitochondria showed degenerative and bizarre changes, with mitochondrial swelling and autolysosome formation. However, the amount of filaments were similar to cells in resting phase.

Co-culturing the cells with IFN-γ and TSH resulted in suppression of the morphological responsiveness to TSH. IFN-γ inhibited TSH-induced microvilli elongation and pseudopod formation. Degenerative changes were similarly observed in the intracytoplasmic organelles except that there were less residual bodies. However, there were no observable changes in the number of cytoplasmic filaments. Furthermore, binucleated cells were again frequently encountered. These, however, were not present in wells treated with control medium or with TSH. Similar findings were observed in cells cultured for either 2 days or 5 days.

DISCUSSION

The role of cellular mechanisms in the pathogenesis of autoimmune thyroid disease has been well described. Though the exact mechanism which results in the activation of T lymphocytes remains elusive, the consequence of the released IFN-γ from the activated T cells on thyroid metabolism is now being appreciated. We and others [4] have previously reported that IFN-γ alone does not affect the basal TG secretion, but in the presence of TSH it suppresses the stimulatory effect of TSH-induced TG

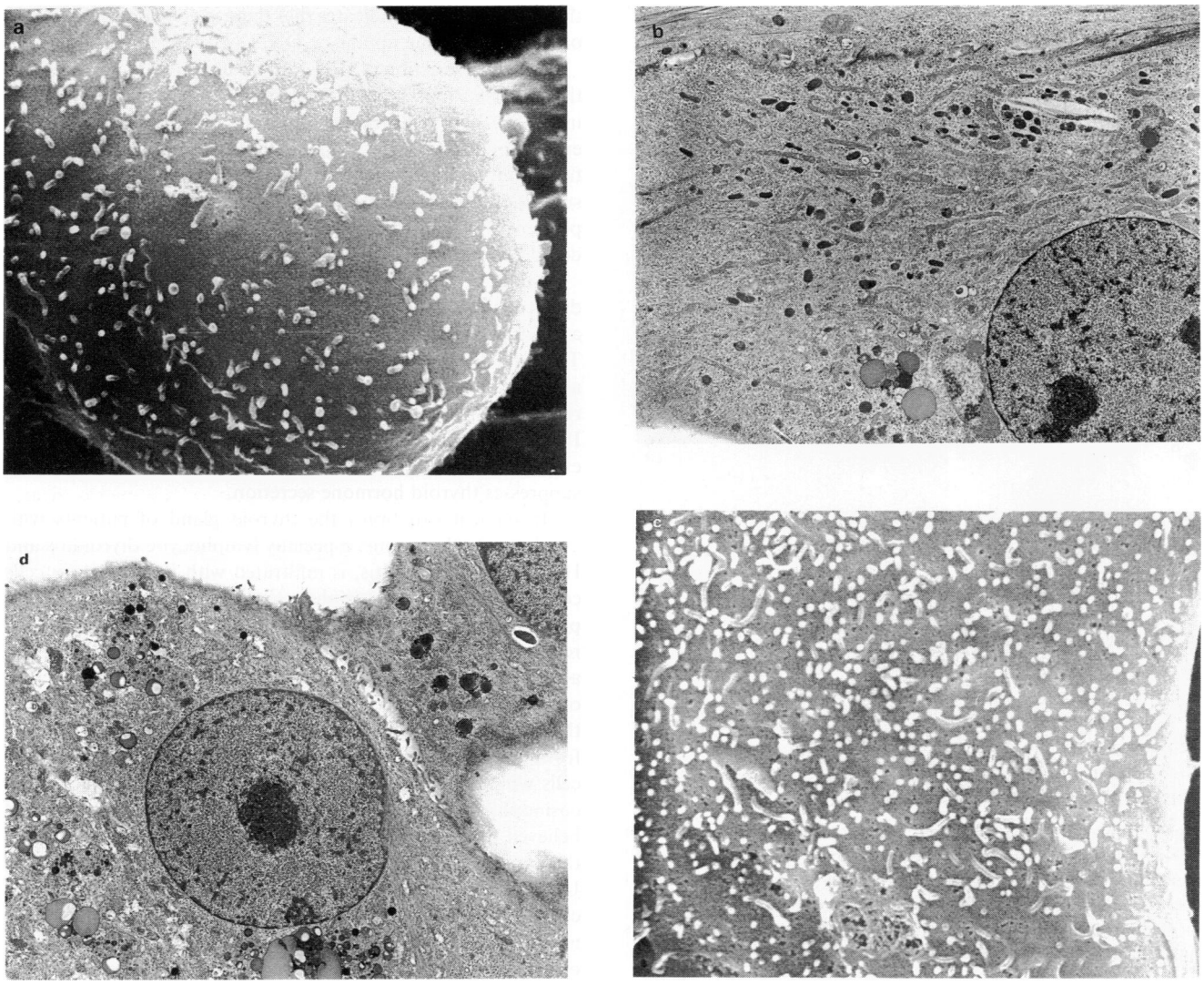

Fig. 1. Human thyrocytes cultured in monolayer in the presence of either control medium (a, b) or TSH 10 mU/ml (c, d) for 2 days. (a) Few short microvilli on the cell surface (SEM). (b) Few intracellular organelles (× 4000). (c) Marked increase in number and length of microvilli with pseudopod formation (SEM). (d) Increase in cytoplasmic organelles and colloid droplets after TSH stimulation (× 4000).

secretion through inhibition of gene transcription. The present study provides further information on the morphological and pathological mechanism of IFN-γ on the thyrocytes.

Our findings suggest that IFN-γ resulted in cytostatic effect on the human thyrocytes in culture. The ultrastructural findings of the thyrocytes were very similar to those observed in patients with autoimmune thyroiditis [5,6]. Thyrocytes of patients with Hashimoto's disease are characterized by a markedly dilatated endoplasmic reticulum, with an increase in lipofuscin and inclusion droplets, as well as in the number of mitochondria resulting in cytoplasmic oxyphilia. Our study similarly revealed that thyrocytes stimulated with IFN-γ disclosed degenerative changes as illustrated by shrinkage of the cell membrane and by the presence of lysosomal granules, laminated figures, autolysosomes and degeneration of mitochondria.

In the presence of TSH, IFN-γ showed marked inhibition of the morphological changes induced by TSH, such as inhibition of TSH-induced microvilli elongation and pseudopod formation. This finding was also reported recently in mouse thyrocytes [7]. Furthermore, we observed that IFN-γ inhibited the hypertrophy of Golgi apparatus and rough-surfaced endoplasmic reticulum induced by TSH stimulation. These differential changes are necessary for colloid reabsorption and thyroid hormone synthesis and secretion [8], as was evidenced by a suppression of TSH-induced TG release into the medium. This explains the association of hypothyroidism in patients who were given IFN-γ therapy [9]. We did not observe any significant change in the amount of cytoplasmic filaments. At the studied dose, IFN-γ did not cause lysis of the thyrocytes, as reflected by the constant amount of cellular DNA content. We further describe for the first time how in the wells treated with IFN-γ with or without the presence of TSH, binucleated cells were frequently seen. These binucleated cells were not present in those cells cultured in control medium or TSH alone. In the

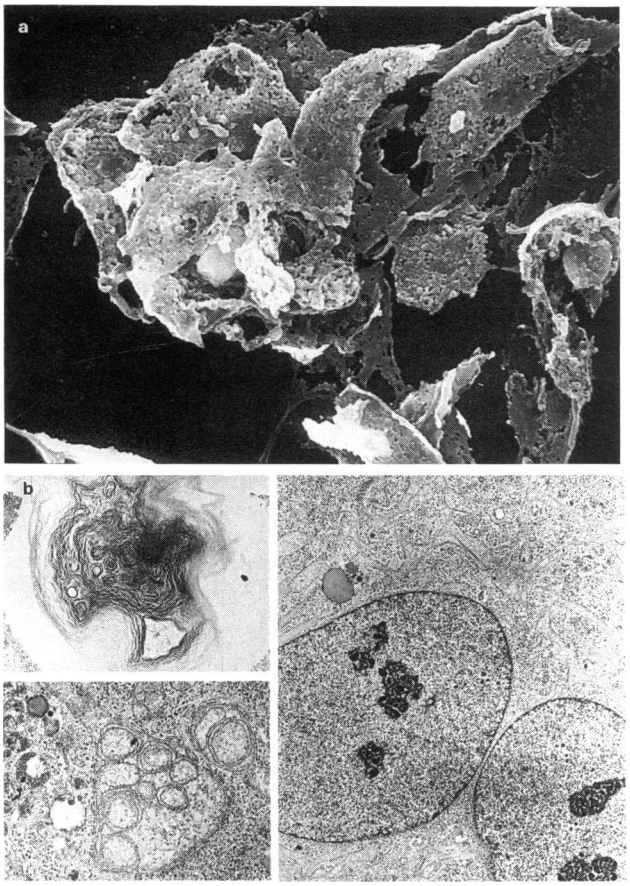

Fig. 2. Human thyrocytes cultured in monolayer and stimulated with IFN-γ 500 U/ml. (a) Marked degeneration with shrinkage of cell membrane (SEM). (b) Cell appeared spindle-shaped, vacuolated and decreased number of intracellular organelles. Cell with two nuclei shown ($\times$ 4000). Degeneration was evidenced by presence of laminated bodies (upper insert, $\times$ 8000) and bizarre mitochondria (lower insert, $\times$ 8000).

present study, the thyrocytes in culture were already confluent in the wells and stopped proliferating due to contact-inhibition before the test agents were added to the medium. This simulates the situation in adult human thyrocytes which have limited proliferation capacity. Thus, we believe that IFN-γ resulted in degeneration and de-differentation of the thyrocytes, which subsequently regained their growth potential and showed attempts at regeneration. However, the amount of DNA per well was not statistically different during the studied period. It had been previously reported that IFN-γ caused inhibition of [^{3}H]-thymidine incorporation in humans [10] and FRTL5-rat thyrocytes [11,12], but all these experiments were performed on dividing cells which did not resemble the situation of normal adult human thyrocytes *in vivo*. Furthermore, the use of [^{3}H]-thymidine incorporation as an index for thyroid growth must be treated with caution [13]. Indeed, it had been reported that although labelling of newly synthesized DNA by [^{3}H]-thymidine was inhibited within a few hours of IFN-γ treatment in L1210 leukaemia cells [14], the incorporation of [^{3}H]-deoxyadenosine into DNA was scarcely altered. Again, in human fibroblast thymidine incorporation was not changed although the cells showed definite slowing of the rate of proliferation after IFN-γ treatment [15]. This indicates that there is no major impairment of either thymidine uptake or DNA synthesis in these cells.

The diverse action of IFN-γ on cellular growth and differentiation of various types of cells is well appreciated. While it inhibited thyroid hormone synthesis and thyroid peroxidase expression in thyrocytes [16], it induced morphological differentiation in neuroblastoma cell lines with increase in cell-specific surface antigen expression and also increase in cytoskeletal proteins [17]. Thus, IFN-γ can result in either differentiation or de-differentiation depending on the cell type studied [18].

Another important role of IFN-γ in autoimmune thyroid diseases is the induction of aberrant expression of MHC Class II antigen by the thyrocytes [19]. This action is further enhanced by TSH which by itself has little effect [20]. Thyroid cell class II antigens participate in activation and amplification of T cell responses, leading to release of more cytokines, including IFN-γ. Released IFN-γ thus further stimulates MHC Class II expression to propagate the inflammatory process and also suppresses thyroid hormone secretion.

In clinical situations, the thyroid gland of patients with autoimmune thyroiditis, especially lymphocytic thyroiditis and Hashimoto's thyroiditis, is infiltrated with inflammatory cells composed mainly of lymphocytes, plasma cells and macrophages [21]. The number and size of the thyroid follicles may be reduced, depending on the severity of the inflammation. The ability of IFN-γ to induce regeneration of the thyrocytes might offer an explanation as to why most patients with lymphocytic thyroiditis would recover from the acute injury and do not suffer from permanent hypothyroidism. Indeed, the presence of giant cells with large hyperchromatic bizarre nuclei and vacuolized eosinophilic cytoplasm observed in autoimmune thyroiditis was believed to represent regenerating thyrocytes after exposure to the lymphokines released by the infiltrating T cells [22,23]. Under EM the cytoplasm of these giant cells, termed Hurthle cells, are filled with a large number of mitochondria representing mitochondrial regeneration [24]. It is believed that the presence of these giant cells represents a transient regenerative stage with compensation to cellular injury, where the surviving thyroid follicular cells try to maintain normal thyroid function after the attack of thyroiditis with increase in number of intracellular organelles that are responsible for protein secretion. Our findings of binucleated cells in IFN-γ-treated thyrocytes strongly support this theory. It is not certain how long this regenerative stage will last and in cases with severe tissue damage, some patients will eventually develop hypothyroidism. Unfortunately, there are no data on the tissue levels of IFN-γ in patients with lymphocytic thyroiditis to correlate with the dosage of IFN-γ employed in the present study. The question arises as to why *in vivo* administration of IFN-γ resulted only in a disease state resembling Hashimoto's thyroiditis [9], and what is the role of IFN-γ in Graves' disease which is caused by TSH receptor antibodies and characterized by hyperthyroidism and thyroid follicular hyperplasia without features of degeneration [25]. A possible explanation for the different biochemical and histological features may be, first, there is enhancement of T cell cytotoxicity in Hashimoto's thyroiditis which is not seen in Graves' disease [26]. Second, the pattern of lymphocytic infiltration of the thyroid gland, which is diffuse in Hashimoto's disease *versus* focal aggregation in Graves' disease, may influence the level of IFN-γ presented to the thyrocytes. Thus in cases of Graves' disease which show similar histologic features

of Hashimoto's thyroiditis, an associated tendency to immunologic destruction of thyroid cells and hypothyroidism is observed [27].

IFN-γ inhibited the functional and morphological response of cultured human thyrocytes to TSH. Furthermore, not only did it not exert cytotoxicity, regeneration with binucleated cells was seen. These observations simulate those findings in patients with lymphocytic thyroiditis and suggest that IFN-γ released by infiltrating T lymphocytes may play an important role in modulating this disease process.

ACKNOWLEDGMENTS

The authors wish to thank Mr Yau wan-lung and Mr Cheung tze-kin for their excellent technical assistance on TEM and SEM preparations, respectively, and Ms Venus Yuen for secretarial assistance.

REFERENCES

1 Kung AWC, Lau KS. Interferon-γ inhibits thyrotropin-induced thyroglobulin gene transcription in cultured human thyrocytes. J Clin Endocrinol Metab 1990; **70**:1512–7.

2 Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 1956; **62**:315–23.

3 Bech K, Madsen SN. Thyroid adenylate cyclase stimulating immunoglobulins in thyroid diseases. Clin Endocrinol 1979; **11**:47–58.

4 Nagayama Y, Izumi M, Ashizawa K *et al.* Inhibitory effect of interferon-γ on the response of human thyrocytes to thyrotropin stimulation: relationship between the response to TSH and the expression of DR antigen. J Clin Endocrinol Metab 1987; **64**:949–53.

5 Neve P. Ultrastructure of the thyroid in de Quervain's subacute granulomatous thyroiditis. Virchows Arch [A] 1970; **351**:87–92.

6 Reidbord HE, Fisher ER. Ultrastructural features of subacute granulomatous thyroiditis and Hashimoto's disease. Am J Clin Pathol 1973; **59**:327–37.

7 Asakawa H, Miyagawa JI, Hanafusa T *et al.* Interferon-γ reduces actin filaments and inhibits thyroid-stimulating hormone-induced formation of microvilli and pseudopods in mouse monolayer thyrocytes. Endocrinology 1990; **127**:325–9.

8 Wetzel BK, Spicer SS, Wollman SH. Changes in fine structure and acid phosphatase localization in rat thyroid cells following thyrotropin administration. J Cell Biol 1965; **25**:593–618.

9 Burman T, Totterman TH, Oberg K, Anders Karlsson F. Thyroid autoimmunity in patients on long term therapy with leucocyte-derived interferon. J Clin Endocrinol Metab 1986; **63**:1086–90.

10 Kraiem Z, Sobel E, Sadeh O, Kinarty A, Lahat N. Effects of γ-interferon on DR antigen expression, growth, 3,5,3′-triiodothyronine secretion, iodide uptake and cyclic adenosine 3′5′-monophosphate accumulation in cultured human thyroid cells. J Clin Endocrinol Metab 1990; **71**:817–24.

11 Zakarija M, Hornicek FJ, Levis S, McKenzie JM. Effects of γ-interferon and tumor necrosis factor on thyroid cells: induction of class II antigen and inhibition of growth stimulation. Mol Cell Endocrinol 1988 **58**:129–36.

12 Misaki M, Tramontano D, Ingbar SH. Effects of rat γ- and non-γ-interferons on the expression of Ia antigen, growth and differentiated functions of FRTL5 cells. Endocrinology 1988; **123**:2849–57.

13 Dumont JE, Roger PP, Ludgate M. Assays for thyroid growth immunoglobulins and their clinical implications: methods, concepts and misconceptions. Endo Rev 1987; **8**:448–52.

14 Brouty-Boye D, Tovey MG. Inhibition by interferon of thymidine uptake in chemostat cultures of L1210 cells. Intervirology 1978; **9**:243–52.

15 Pfeffer LM, Morphy JS, Tamm I. Exp Cell Res 1979; **121**:111–20.

16 Ashizawa K, Yamashita S, Nagayama Y *et al.* Interferon-γ inhibits thyrotropin-induced thyroidal peroxidase gene expression in cultured human thyrocytes. J Clin Endocrinol Metab 1989; **69**:475–7.

17 Parodi MT, Cornaglia-Ferraris P, Ponzoni M. Effects of γ-interferon on the growth, morphology, and membrane and cytoskeletal proteins expression of Lan-1 cells. Experimental Cell Res 1989; **185**:327–341.

18 Clemans MJ, McNurlan MA. Regulation of cell proliferation and differentiation by interferons. Biochem J 1985; **226**:345–60.

19 Bottazzo GF, Todd I, Mirakian R, Belfiore A, Pujol-Borrell R. Organ-specific autoimmunity: a 1986 overview. Immunol Rev 1986; **94**:137–69.

20 Todd I, Pujol-Borrell R, Hammond LJ, McNally JM, Feldmann M, Bottazzo GF. Enhancement of thyrocyte HLA class II expression by thyroid stimulating hormone. Clin Exp Immunol 1987;**69**:524–31.

21 Volpe R. Hashimoto's thyroiditis. Compr Ther 1977; **3**:68–76.

22 Lindsay S, Dailey ME, Fridlander J *et al.* Chronic thyroiditis: a clinical and pathological study of 354 patients. J Clin Endocrinol 1952; **12**:1578–600.

23 Stein AA, Hernandez I, McClintock JC. Subacute granulomatous thyroiditis: a clinicopathologic review. Ann Surg 1961 **153**:149–56.

24 Bastenie PA, Ermans AM. Thyroiditis and thyroid function. International Series of Monographs in Pure and Applied Biology. Modern Trends in Physiological Sciences. vol. 36. Oxford: Pergamon Press, 1972:172–6.

25 Oertel JE, LiVolsi VA. Pathology of thyroid diseases. In: Ingbar SH, Braverman LE, eds. Werner's the thyroid, a fundamental and clinical text. Philadelphia: Lippincott, 1986:651–86.

26 Hiromatsu Y, Fukazawa H, Wall JR. Cytotoxic mechanisms in autoimmune thyroid disorders and thyroid-associated ophthalmopathy. Clin Endocrinol Metab North Am 1987; **16**:267–86.

27 Bellanti JA. Immunologically mediated diseases. In: Bellanti JA, ed. Immunology II. Philadelphia: Saunders, 1978: 471–86.