

Interleukin-1 affects the function of cultured human thyroid cells

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Cytokines are peptide hormones essential for cellular communication in the immune response. The purpose of this study was to investigate the influence of cytokines, especially recombinant interleukin 1 β (rIL-1 β), on human thyroid cells. Thyroglobulin (Tg) was measured by a double antibody radioimmunoassay, and cyclic AMP (cAMP) by a competitive protein binding assay. Supernatants from unstimulated and phytohaemagglutinin-stimulated blood mononuclear cells were added to human thyroid cells cultured in monolayers. A dose-dependent inhibition of the secretion of Tg and cAMP was demonstrated. Both subcultured and primary cultured cells incubated with rIL-1 β at pharmacological levels (10^{-1} – 10^2 U/ml) exhibited an inhibition of Tg and cAMP secretion, while at physiological levels (10^{-5} – 10^{-3} U/ml), the secretion of Tg was enhanced. The similar stimulation of cAMP was demonstrated in subcultures. These *in vitro* studies suggest that IL-1 β may play a role in the pathogenesis of autoimmune thyroid diseases. Further, the stimulations at low concentrations indicate that IL-1 β may regulate the function of the thyroid gland under physiological conditions.

Key words: autoimmunity; cAMP; interleukin-1; thyrocytes; thyroglobulin

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CLINICAL ASPECTS

This study of autoimmune mechanisms in thyroid diseases, evaluated by morphological and functional cell studies on thyrocytes, is of general importance in the understanding of the pathogenesis of autoimmune diseases. The results should be evaluated in connection with studies of the pathogenesis in other endocrine diseases and in other autoimmune diseases in general. The clarification of the pathogenetic pathways in autoimmune diseases will open the possibilities of therapy.

Activated mononuclear cells secrete a number of biologically potent antigen-nonspecific hormones, termed cytokines, which are essential for cellular communications during immunological and inflammatory responses. Interleukin 1 (IL-1) is a T- and B-lymphocyte-activating cytokine released primarily by antigen-presenting macrophages (5, 9). In addition to its lymphocyte-activation properties, IL-1 has a broad spectrum of other activities,

including the ability to alter neuroendocrine and metabolic functions (5, 9).

Recently, IL-1-containing supernatants of activated human blood mononuclear cells (MNC), purified native IL-1, as well as recombinant IL-1 β (rIL-1 β), were shown to directly affect the function and structure of beta-cells in pancreatic islets of Langerhans (6, 15, 16, 17). The secretion of insulin was inhibited, and the content of insulin was

decreased in parallel with the decreasing dose. At low concentrations, IL-1 significantly increased the release of insulin (6, 20).

The purpose of this study was to investigate the influence of cytokines, and in particular of IL-1, on another endocrine target cell in order to elucidate a possible participation of IL-1 and/or other cytokines in the pathogenesis of thyroid diseases. We found that IL-1 at pharmacological levels inhibited and at physiological concentrations stimulated the release of thyroglobulin (Tg) and cyclic AMP (cAMP) from *in vitro* cultures of thyroid cells.

MATERIAL AND METHODS

Thyroid cell culture

Fresh human paraadenomatous thyroid tissue obtained from 5 patients during thyroid surgery was transported under sterile conditions to the laboratory in ice-cold phosphate buffered saline (Dulbecco's). The tissue was cleaned free of connective and fat tissue, aseptically minced into small pieces, and prepared as reported by Reader et al. (19) with minor modifications. The pieces were washed in a calcium- and magnesium-free phosphate buffer (pH 7.4) containing 2 g/l sodium bicarbonate (Dulbecco's formula, Flow Labs, Fredensborg, Denmark), and then digested with 1 mg/ml collagenase I (Sigma, Rødovre, Denmark)/2.4 U/ml dispase II (Boehringer Mannheim, Ercopharm A/S, Herlev, Denmark) at 37°C for 75 min. After filtration and centrifugation at 100 g for 5 min two times, the pellet was resuspended in Eagle's Minimum Essential Medium modified with Earle's salts (Flow Labs) supplemented with 10% heat-inactivated fetal calf serum (Flow Labs), L-glutamin (2 mmol/l, Flow Labs) and antibiotics (100 U/ml of penicillin, 100 µg/ml of streptomycin). The cells were cultured in a humidified air atmosphere, containing 5% CO₂ in Nunclon multidish plates (24 wells, each 1.9 cm²) or in flasks (25 cm²/50ml), (Nunc, Roskilde, Denmark). The cultured cell suspensions of 2 × 10⁵ cells per 1 ml in each well

formed confluent layers within 3–4 days. The confluent layers in the flasks were washed in a calcium- and magnesium-free phosphate buffer and covered with a thin liquid film of a Trypsin/EDTA (0.05%/0.02%) solution (Flow Labs). The cell cultures were then incubated at 37°C for 5 min, washed and subcultured in the serum-containing culture medium in the multidish plates.

Characterization of the cell culture

Evidence that the monolayers consisted of thyrocytes was ascertained by immunohistochemistry using antihuman-thyroglobulin antiserum produced in a rabbit (11). The specimens were fixed in acetone and antihuman-thyroglobulin antiserum was applied, whereafter they were stained by an indirect immunoperoxidase technique (7). The antiserum was diluted 1:200 and 1:400.

The function of both primary and subcultured thyroid cell monolayers was investigated after stimulation with TSH (Thytropar® Armour Pharmaceutical Company Ltd, Eastbourne, U.K.) at a concentration of 0.1 mU/ml and/or insulin (Calbiochem, Hoechst, Rødovre, Denmark) in a concentration of 10 µg/ml and 0.5 mmol/l 3-isobutyl-L-methyl-xanthine (IBMX). Kinetic studies of the response of cAMP (measured by competitive protein-binding assay (3)) and Tg (measured by a double antibody radioimmunoassay (12)) were investigated by removing 150 µl of supernatants 0.5, 1, 1.5, 2, 3, 5, 24, 48, 72 and 96 h after adding TSH. After 4 days of culture, the supernatants were removed and the cellular contents of DNA were determined by the diphenylamine method (8). The supernatants were kept at 20°C until measurements of Tg and cAMP.

Cytokines

Crude cytokine preparations and recombinant human IL-1. MNC were isolated from healthy donors, pulsed for 1 h at 37°C. The supernatants were isolated from the cells by centrifugation, dialysed, lyophilised and stored at -20°C. The control supernatants contained 120 U/ml of

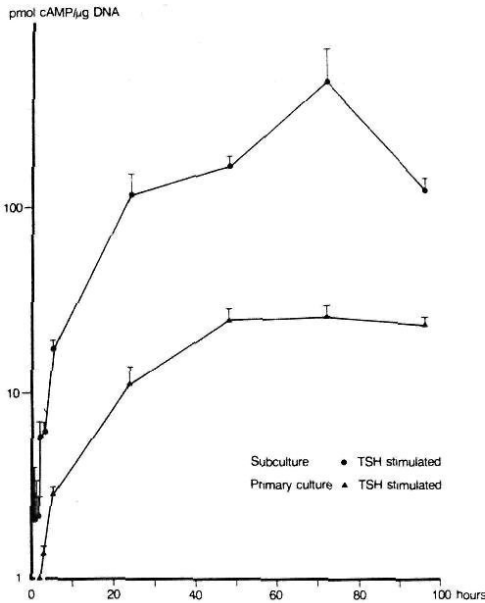


Fig. 1. Accumulation of cyclic AMP (cAMP) in TSH-stimulated human thyroid cultures. cAMP content is expressed as pmol cAMP/ μ g DNA in supernatants from thyroid primary cultures ($\blacktriangle$) or subcultures ($\bullet$) of the same origin. Mean values + SD are shown ($n = 4$). Abscissa: time of culture.

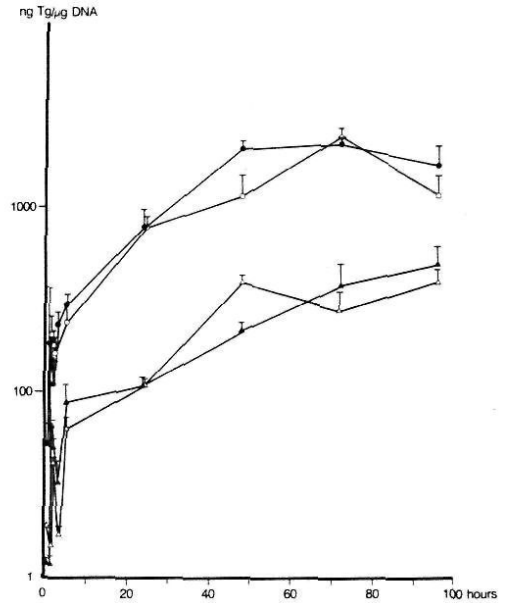


Fig. 2. Accumulation of thyroglobulin (Tg) in unstimulated (open symbols) and TSH-stimulated (closed symbols) thyroid primary cultures (triangles) and subcultured cells (circles) from the same donor. Mean values + SD are shown ($n = 4$). Abscissa: time of culture.

IL-1 (due to contamination of culture medium with low concentrations of endotoxin). The supernatants of PHA-activated MNC contained 1600 U/ml of IL-1.

The cDNA for human IL-1 β (2) was expressed in *E. coli* as a 24,000 MW peptide (N-terminus at position 71) and then further processed by limited proteolytic cleavage to a 17,000 MW peptide with the N-terminus at position 112 as previously described (2).

IL-1 activities were measured by a mouse thymocyte co-stimulatory assay (10) and expressed in units (U). One unit of IL-1 is the amount of IL-1 that doubles the background proliferation of the thymocytes to a sub-mitogenic dose of PHA (20 mg/l).

Effect of cytokines on thyrocytes

Human primary or subcultured monolayers of thyrocytes were incubated with supernatants

from unstimulated or PHA-stimulated MNC or human rIL-1 β .

Tg and cAMP in the supernatants were measured on days 2 and 4.

Statistical method

The Student's t-test was used with a chosen significance level of 5%.

RESULTS

Cells

Immune peroxidase staining, using antibodies to human Tg, showed that Tg-containing cells comprised the majority of the monolayers.

Influence of TSH stimulation

The production of cAMP after 4 days of cul-

ture with TSH was increased in both primary and subcultured thyroid cells when compared with unstimulated cultures ($P < 0.001$, $n = 12$ and 13). This stimulation was not influenced by insulin. Detectable levels of cAMP were demonstrated in subcultured, TSH-stimulated cells after 30 min, whereas significant levels were measurable after 90 min in primary cultures. Peak concentrations were seen after 3 days in both types of cultures, but the levels were higher in those of subcultured cells (Fig. 1).

The secretion of Tg was not increased by TSH and was independent of insulin in both primary cultures and subcultures. The concentration of Tg increased gradually in both unstimulated and TSH stimulated cultures to maximal levels after 3 days (Fig. 2). After 4 days of culture with or without TSH the Tg level was 748 ± 322 (mean + SD) ng/ μ g DNA ($n = 14$) versus 736 ± 290 ng/ μ g DNA ($n = 12$) (not significant, (NS)). There was a significant increase in cAMP after TSH stimulation (28.0 ± 4 versus 0.5 ± 0.4 pmol cAMP/ μ g DNA, $n = 4$, $2 P < 0.001$). Table 1 shows the basal and stimulated levels of cAMP and Tg in primary and subcultured thyroid cells obtained from the same gland. It appears that the levels as well as the TSH sensitivity are higher in the subcultures.

Influence of supernatants from MNC

When supernatants from both unstimulated and PHA-stimulated MNC were added undiluted and at dilutions up to 1:1000, a dose-dependent inhibition of the TSH-stimulated Tg-secretion was seen. The production of cAMP was also reduced, but a marked dose-dependent reduction was seen only at higher concentrations (at dilutions up to 1:100), (data not shown).

Influence of rIL-1 β

We next studied the influence of purified rIL-1 β on Tg secretion and cAMP production in primary and subcultured thyroid cultures. rIL-1 β , at concentrations above 1 U/ml, inhibited both unstimulated and TSH-stimulated Tg secretions as well as TSH-stimulated cAMP production up to 75% ($2 P < 0.001$). In contrast, rIL-1 β at low concentrations, around 0.0001 U/ml significantly stimulated Tg secretion up to 190% ($2 P < 0.01$) (Fig. 3). This stimulation was found in both subcultured and primary cultures, whereas a similar stimulation of cAMP production was demonstrated only in subcultures ($2 P < 0.01$) (Fig. 4). The dose-response curves showing the effect of purified rIL-1 β on Tg-secretion corresponded closely to those obtained when using MNC supernatants

Table 1

Basal and TSH-stimulated (100 mU/l) levels of cyclic AMP (cAMP) and thyroglobulin (Tg) in primary and subcultured thyroid cells from the same donor cultured in the presence or absence of insulin (10 μ g/ml)

Primary culture	+ Insulin		- Insulin	
	- TSH	+ TSH	- TSH	+ TSH
pmol cAMP/ μ g DNA	2.8 ± 0	8.4 ± 5.7	0.0	13.4 ± 0
	NS ($n = 4$)		NS ($n = 4$)	
ng Tg/ μ g DNA	423 ± 132	376 ± 0	390 ± 217	240 ± 11
Subcultured cells	- TSH	+ TSH	- TSH	+ TSH
	- TSH	+ TSH	- TSH	+ TSH
pmol cAMP/ μ g DNA	4.2 ± 1.7	30.9 ± 7.9	6.6 ± 2.9	52.9 ± 22.7
	$2 P < 0.001$ ($n = 5$)		$2 P < 0.025$ ($n = 3$)	
ng Tg/ μ g DNA	660 ± 194	722 ± 133	730 ± 162	555 ± 78
	NS ($n = 4$)		NS ($n = 4$)	

NS: not significant, comparing TSH-stimulated and unstimulated cultures ($P > 0.05$).

Results are shown as mean values $\pm$ SD.

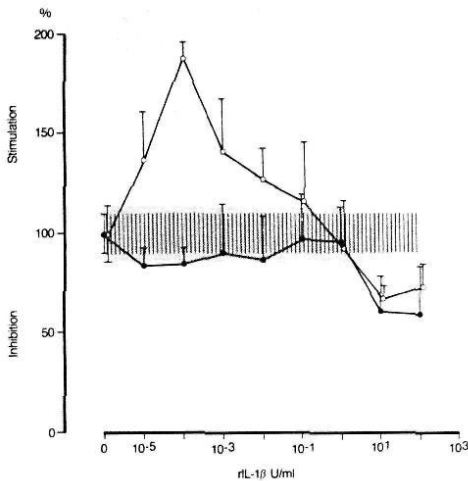


Fig. 3. Changes in thyroglobulin (Tg) (○) and cyclic AMP (cAMP) (●) secretion from thyroid monolayers ($n = 4$ and 13) stimulated for 4 days with TSH (100 mU/l) in the presence of recombinant interleukin 1 β (rIL-1 β) compared with TSH-stimulated cultures without addition of rIL-1 β . Mean values $\pm$ SD are shown. The lined (cAMP) and the hatched (Tg) areas indicate mean values $\pm$ SD found in control cultures without rIL-1 β . The activity of rIL-1 β is measured by a mouse thymocyte co-stimulatory assay (10).

if calculated on the basis of their concentrations of IL-1.

The maximal inhibitory effect of rIL-1 β on the elaboration of cAMP was demonstrated after 2 days, whereas the Tg response was maximal after 4 days of culture (Fig. 4).

Removal of rIL-1 β and readdition of TSH and insulin abolished the inhibition of cAMP secretion after 3 more days of culture. This indicates that rIL-1 β acted in a non-cytotoxic manner. The Tg-secretion, however, remained low under these circumstances (data not shown).

DISCUSSION

Characterization of the thyroid cell cultures

The release of Tg into the medium was not influenced by the addition of TSH to the human thyroid cells. This was in accordance with the results found by Lissitzky et al. (14)

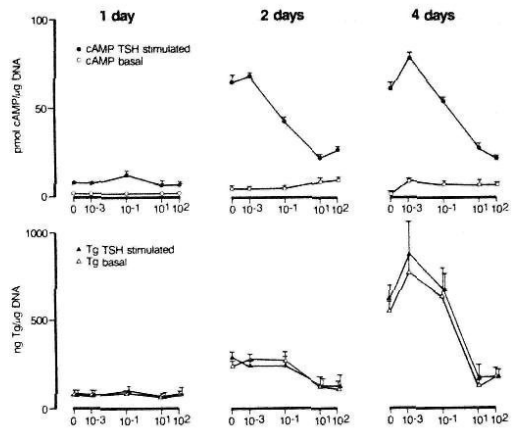


Fig. 4. Influence of recombinant interleukin 1 β (rIL-1 β) up to 10² U/ml (abscissa), on unstimulated (open symbols) and TSH-stimulated (100 mU/l) (closed symbols) subcultured thyroid cells on secretion of cyclic AMP (cAMP) in pmol/ μ g DNA (circles, upper panels) and thyroglobulin (Tg) in ng/ μ g DNA (triangles, lower panels) after incubation for 1, 2, or 4 days. Mean values $\pm$ SD are shown ($n = 4$).

using porcine thyroid cells. A stimulation of the cAMP response to TSH was in this study found in both primary and subcultured human thyroid cells. This stimulatory effect on cAMP secretion was also demonstrated in monolayers of dog thyroid cells (18), in porcine thyroid cells cultured both in monolayers (19) and in follicles (3), and in the continuous culture line of rat thyroid (FRTL5) cells (22).

We found a maximal stimulation of cultured human thyroid cells after 3 days of TSH-stimulation (100 mU/l) in contrast to findings with cultured dog thyroid cells, where maximal cAMP levels were achieved after 10–15 min of TSH stimulation (50 μ U/l) (18). This discrepancy probably can not be explained by the moderately different concentrations of TSH, but it could be due to different sensitivities or culture conditions of the two species of thyroid cells (dog and human).

In contrast to FRTL5 cells (1), insulin does not appear to influence the cAMP or Tg response in cultured human thyroid cells.

The levels of Tg and cAMP per μ g DNA were higher in subcultures compared with primary cell cultures, probably as a result of a

more pure culture of thyrocytes. The subcultures were therefore preferred for the *in vitro* studies of thyroid cell function.

Possible involvement of IL-1 in pathogenesis of autoimmune thyroid diseases

Lymphocytes infiltrate the thyroid gland in Hashimoto's and in Graves' disease. This infiltration is only one of several features indicating autoimmune pathogenetic mechanisms in these diseases. The purpose of the present study was to further explore this hypothesis.

IL-1 production is considered a result of an immune reaction, where an antigen initiates a series of cellular and humoral interactions leading to activation of specifically reactive T-lymphocytes. This T-cell activation is dependent upon antigen presentation and IL-1 production by the antigen-presenting cells, usually macrophages. As a result, the T-cells elaborate macrophage-activating factors, including interferon- γ , which in an autocrine circle increase the production and secretion of IL-1 (5).

The predominant species of human IL-1, termed IL-1 β , is a potent suppressor of insulin production by rat and human pancreatic β -cells, most likely as a result of a direct and selective cytotoxic effect on the β -cells (6, 16, 17). The present study demonstrates that rIL-1 β also inhibits Tg and cAMP secretion from cultured human thyroid cells indicating that IL-1 may indeed play a role also in autoimmune endocrine thyroiditis. However, on the thyrocytes IL-1 β -mediated inhibition of cAMP secretion was reversible, indicating no evidence of a cytotoxic effect of IL-1.

The stimulatory effect on Tg and cAMP release observed at very low concentrations of rIL-1 β corresponds to the stimulatory effect of minute amounts of rIL-1 β on insulin production by rat pancreatic β -cells (20). In FRTL5 cells, IL-1 has also been shown to augment DNA synthesis and cell proliferation (21), but it did not influence the adenylate cyclase. Because low concentrations of IL-1 may accumulate the endocrine tissues by diffusion from the blood during conditions of "stress" we believe that IL-1 under these circumstances may exert a

physiological regulatory role by stimulating adenylate cyclase and potentiating Tg and insulin production.

In conclusion, the findings suggest that IL-1 may play a role in the molecular mechanisms contributing to dysfunction in autoimmune thyroid diseases and that IL-1 under physiological conditions, may regulate thyroid function.

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