

## REVIEW

## Role of cytokines in the pathogenesis of the euthyroid sick syndrome

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### Introduction

Nonthyroidal illness (NTI) is associated with complex changes in thyroid function tests and thyroid regulation known as the euthyroid sick syndrome (ESS) (1). The most common abnormality is a reduction in serum total triiodothyronine (TT<sub>3</sub>) and, to a lesser extent, free T<sub>3</sub> (FT<sub>3</sub>) concentrations (2). Kinetic studies showed that the daily production rate (PR) of T<sub>3</sub> is decreased, while its clearance is unchanged in NTI (3). The reduction in serum TT<sub>3</sub> concentration is usually accompanied by an increase in serum reverse T<sub>3</sub> (rT<sub>3</sub>) concentration (1), although the latter is normal in patients with chronic renal failure (4), AIDS (5), or traumatic brain injury (6). PR of rT<sub>3</sub> is unchanged, but its clearance is reduced (3). These changes in serum TT<sub>3</sub> and rT<sub>3</sub> concentrations are related to inhibition of the activity of type I 5'-deiodinase (5'-DI), the enzyme catalyzing deiodination of thyroxine (T<sub>4</sub>) to T<sub>3</sub> and of rT<sub>3</sub> to 3,3'-diiodothyronine (7). Serum thyrotropin (TSH) concentration is usually normal, although suppressed values may be found in a minority of patients (1). In addition, a decrease in the nocturnal surge of TSH has been consistently reported in patients with NTI (8–12). Abnormalities of TSH glycosylation causing its decreased biological activity were also described (13). The decrease in serum TSH secretion is in some instances related to the use of dopamine and glucocorticoids in critically ill patients (14).

The pathogenesis of changes in serum thyroid hormone and TSH concentrations leading to ESS is not completely understood. Reduced T<sub>3</sub> generation in peripheral tissue may be related not only to a decreased 5'-DI activity, but also to decreased T<sub>4</sub> transport into tissues (15). Substances, such as 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) and indoxyl-sulfate, which are increased in chronic renal failure, and bilirubin and free fatty acids (FFA), which are increased in other NTI, were reported to decrease T<sub>4</sub> uptake in rat liver tissue (16, 17). Interestingly, CMPF and indoxyl-sulfate did not affect TSH secretion in cultured rat anterior pituitary cells (18).

Recently, particular attention was focused on the role of cytokines in the pathogenesis of ESS. Cytokines are multifunctional molecules with different biological effects and target cells, which can exert autocrine (on

the same cells that secrete them) and paracrine (on adjacent cells), but also endocrine (on distant cells) actions (19). They are usually produced in response to inflammation, infections and cellular injury, and their physiological role remains to be clarified (19). In general, cytokines act through binding to specific cell-surface receptors that were demonstrated also in thyrocytes (20, 21). In addition, thyrocytes synthesize and release cytokines, which might be involved in autocrine or paracrine regulation of thyroid function (22–32). The importance of cytokines in thyroid pathophysiology was recently underscored (Fig. 1). They participate in the pathogenesis of thyroid autoimmune disease by contributing to the growth and differentiation of B and T cells, by inducing expression of HLA class II antigens and adhesion molecules, by recruiting and activating immune cells and by modulating the course of the disease (33). Interleukin-6 (IL-6) and possibly other cytokines are useful markers of thyroid-destructive processes (34). Cytokines have a role in the pathogenesis of Graves' ophthalmopathy, since they induce orbital fibroblast proliferation and glycosaminoglycan production, and expression of HLA class II antigens, adhesion molecules and heat shock proteins in orbital fibroblasts (35). Numerous studies *in vitro* and *in vivo* (both in animals and humans) were carried out in recent years on the effects of cytokines on thyroid function. Since the number of published papers on this issue is very large, in this review we analyzed the most crucial studies to discuss the role of cytokines in the pathogenesis of ESS.

### Effects of cytokines on thyroid function *in vitro*

Several effects of cytokines that might be relevant in the pathogenesis of ESS were demonstrated in *in vitro* systems (Table 1). Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) had no effect on iodide uptake under basal conditions in FRTL-5 cells (21), but it reduced TSH-stimulated iodide uptake in the same cell system (21, 36), as well as in four thyroid cancer cell lines (21). This action appeared not to be related to inhibition of TSH binding to its receptor or to be mediated by the phospholipase

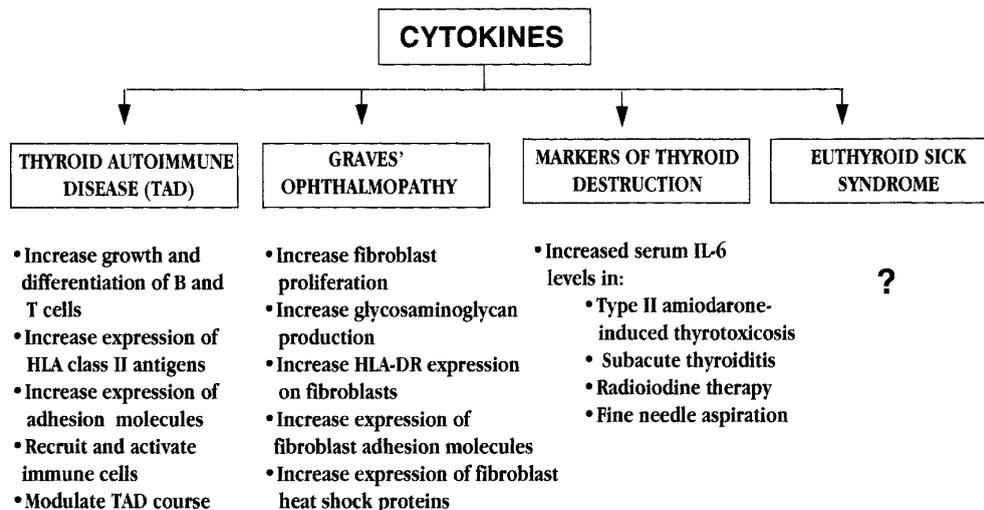


Figure 1 Involvement of cytokines in thyroid pathophysiology.

$A_2$ -arachidonic acid pathway or by  $H_2O_2$ , since inhibitors of the phospholipase  $A_2$ -arachidonic acid pathway (promethacin, indomethacin) or the  $H_2O_2$  scavenger, catalase, did not block the  $TNF-\alpha$  effect (37). Likewise, IL-1 decreased both basal and TSH-stimulated iodide uptake in FRTL-5 cells (37). Less clear results were obtained using a porcine thyroid cell system, in which IL-1 $\alpha$  enhanced iodination after 1 h and 18 h exposure, but decreased it, overcoming TSH stimulation, after 42 h (38). Likewise, in intact porcine thyroid follicles, IL-1 $\beta$  decreased iodide uptake with no effect on proliferation or cAMP formation (39). Conflicting results were reported as to the effects of interferon- $\gamma$  (IFN- $\gamma$ ). An increase in both basal and TSH-stimulated iodide uptake was reported in FRTL-5 cells (36, 40), whereas an inhibition of TSH-stimulated iodide uptake was observed in human thyrocytes after 5–7 days of culture (39, 40). This issue is further complicated by the observation that  $TNF-\alpha$  potentiated the increase of TSH-stimulated iodide uptake caused by IFN- $\gamma$  in FRTL-5 cells (36) and the inhibition of TSH-stimulated iodide uptake produced by the same cytokine in human thyrocytes (41, 42) (Table 1).

Inhibition of thyroglobulin (Tg) synthesis by cytokines was reported. In cultured human thyrocytes IL-1 $\alpha$  and IL-1 $\beta$  decreased TSH-stimulated Tg mRNA levels, although no effect was apparent on basal Tg mRNA levels (43) (Table 1). At variance, other reports showed that the reduction in the synthesis of Tg caused by both IL-1 $\alpha$  (44) and  $TNF-\alpha$  (45, 46) in human thyrocytes was associated with a decrease in cAMP production. Other studies demonstrated that IL-6 had only marginal effects on Tg synthesis in cultured human thyrocytes, suggesting that IL-1 effects are not mediated by IL-6 induction (47). An inhibitory effect on TSH-stimulated Tg synthesis and secretion was also produced by IFN- $\gamma$

both in cultured human thyrocytes (48) and in FRTL-5 cells (49) (Table 1). Similar inhibition of TSH-stimulated Tg secretion was caused by IFN- $\gamma$  in thyrocytes from Graves' disease patients (50).

Several studies documented the effects of cytokines on thyroid hormone synthesis and secretion. IL-1 $\alpha$  and IL-1 $\beta$  caused a dose-dependent decrease in thyroid peroxidase (TPO) mRNA levels in cultured human thyrocytes from patients with Graves' disease (51) (Table 1). Similar results were obtained, using the same cell system, with IL-6 (52) and IFN- $\gamma$  (53). The latter cytokine also reduced the basal and TSH-stimulated TPO content of cultured human thyrocytes (54). In a human thyrocyte system, IL-1 $\alpha$  and IL-1 $\beta$ , as well as  $TNF-\alpha$  and IFN- $\gamma$ , decreased [ $^{125}I$ ]iodothyronine release in a dose-dependent manner (42). A decrease in TSH-stimulated  $T_3$  secretion was observed in cultured human thyrocytes incubated with IFN- $\gamma$  (41), IL-1 (42), or  $TNF-\alpha$  (42) (Table 1). IL-6 also inhibited TSH-stimulated  $T_3$  secretion in cultured human thyrocytes (52), but in other studies this effect of IL-6 could be obtained, using human thyroid follicles, only in the presence of soluble IL-6 receptor, which binds the cytokine and potentiates its action (55) (Table 1).

Conflicting results were reported on the growth effects of cytokines. An increase in tritiated thymidine incorporation into human thyrocytes and FRTL-5 cells (23, 43) was caused by IL-1, but the opposite effect occurred in the presence of TSH (56) (Table 1). This inhibitory effect of IL-1 in the presence of TSH was also reported in papillary thyroid carcinoma cells (57). Inhibition of thyroid cell proliferation might be mediated by IL-1-stimulated prostaglandin  $E_2$  production (23). In different studies IFN- $\gamma$  caused either an increase (36, 49) or a decrease (58) in tritiated thymidine incorporation, IFN- $\gamma$  decreased (41, 59) and  $TNF-\alpha$  stimulated (58) proliferation of human thyrocytes (Table 1). Cell

**Table 1** Effects of cytokines on thyroid function *in vitro*.

Parameter	Cytokine	Cell system	Effect	Reference
Iodide uptake	TNF- $\alpha$ *	FRTL-5	Decreased**	21, 23
		Thyroid cancer	Decreased**	21
	IL-1	FRTL-5	Decreased**	37
		Porcine thyrocytes	Increased/decreased***	38
	IFN- $\gamma$	Porcine thyroid follicle	Decreased	39
		FRTL-5	Increased	36, 40
	Human thyrocytes	Decreased**	41, 42	
Thyroglobulin synthesis	IL-1 $\alpha$	Human thyrocytes	Decreased mRNA	43
		Human thyrocytes	Decreased mRNA	43
	IL-6	Human thyrocytes	No effect	47
		Human thyrocytes	Decreased mRNA	48
	IFN- $\gamma$	FRTL-5	Decreased mRNA	49
		Graves' thyrocytes	Decreased	50
		Human thyrocytes	Decreased	45, 46
Iodination	IL-1 $\alpha$	Graves' thyrocytes	Decreased TPO mRNA	51
		Graves' thyrocytes	Decreased TPO mRNA	51
	IL-6	Graves' thyrocytes	Decreased TPO mRNA	52
		Graves' thyrocytes	Decreased TPO mRNA	53
	IFN- $\gamma$	Human thyrocytes	Decreased TPO content	54
Thyroid hormone secretion	IL-1	Human thyrocytes	Decreased T <sub>3</sub> secretion**	42
		Human thyrocytes	Decreased T <sub>3</sub> secretion**	52
	IL-6****	Human thyroid follicle	Decreased T <sub>3</sub> secretion*****	55
		Human thyrocytes	Decreased T <sub>3</sub> secretion**	42
	IFN- $\gamma$	Human thyrocytes	Decreased T <sub>3</sub> secretion**	41
Growth effects	IL-1	Human thyrocytes	Increased thymidine uptake	43
		FRTL-5	Increased thymidine uptake	23
	IFN- $\gamma$	FRTL-5	Increased thymidine uptake	36, 49
		Human thyrocytes	Decreased thymidine uptake	58
	TNF- $\alpha$	Human thyrocytes	Decreased proliferation	41, 59
		Human thyrocytes	Increased proliferation	58
TSH synthesis and secretion	IL-1 $\beta$	Rat pituitary cells	Decreased TSH*****	60
		Dispersed pituitary cells	Increased TSH	62
	TNF- $\alpha$	Rat pituitary cells	Decreased TSH	60
Thyroid hormone binding proteins	IL-6	Hep G2	Decreased TBG, TTR, albumin	63
	TNF- $\alpha$	Human, mouse hepatocytes	Decreased albumin	66

\* TNF- $\alpha$  potentiated IFN- $\gamma$  increase of TSH-stimulated iodide uptake in FRTL-5 cells and IFN- $\gamma$  decrease of TSH-stimulated iodide uptake in human thyrocytes.

\*\* Effective only on TSH-stimulated iodide uptake.

\*\*\* Increased after 1 h or 18 h exposure, decreased after 42 h exposure.

\*\*\*\* In human thyroid follicles this effect was obtained only in the presence of soluble IL-6 receptor (55).

\*\*\*\*\* Only in the presence of soluble IL-6 receptor.

\*\*\*\*\* No effect of TRH-stimulated TSH release (60); possibly mediated by increased somatostatin release (61).

aging enhanced the sensitivity of the cells to the cytotoxic effects of TNF- $\alpha$  (58).

With regard to regulation of TSH synthesis and secretion, in rat anterior pituitary cells IL-1 $\beta$  and TNF- $\alpha$  caused a significant decline in TSH release without affecting the release of other pituitary hormones (60) (Table 1). Thyrotropin-releasing hormone (TRH)-induced TSH release was not affected by IL-1 $\beta$ , suggesting that TRH might overcome the inhibitory effect of the cytokine (60); in addition, the pituitary uptake of radioiodinated thyroid hormones did not change in the presence of IL-1 $\beta$  (60), implying that the cytokine effect might not be mediated by an increased uptake (and inhibitory effect) of thyroid hormones. Other studies postulated that cytokines, in particular IL-1 $\beta$ , might exert their effect on TSH release indirectly,

through an enhancement of potassium-stimulated release of somatostatin from the hypothalamus (61). Reported results are not unequivocal, since IL-1 $\beta$  was also reported to stimulate TSH release from dispersed anterior pituitary cells (62).

Cytokines can also affect the synthesis and release of thyroid hormone-binding proteins. In human hepatoblastoma-derived (Hep G2) cells, IL-6 caused a reduction in the synthesis of T<sub>4</sub>-binding globulin (TBG), transthyretin (TTR) and albumin, acting at transcriptional levels (63) (Table 1). Interestingly, in the same cell system, IL-6 caused a decrease also in corticosteroid-binding globulin (CBG) synthesis, acting at a post-transcriptional level, probably through a decreased stability of CBG mRNA (64). Albumin gene expression was reduced also by TNF- $\alpha$  in humans and mice (65,

66) (Table 1). It is worth noting that in Hep G2 cells IL-6 stimulated the synthesis of antitrypsin and antichymotrypsin, two major acute phase proteins showing a high degree of homology with TBG (63). There is no evidence that changes of thyroid function tests in NTI may be attributed to the effects of acute phase proteins.

In summary, although to some extent conflicting due to different cell systems and experimental conditions, *in vitro* studies provided a large body of evidence suggesting that cytokines may influence thyroid function either directly or indirectly. All steps of thyroid hormone synthesis, from iodide uptake to thyroid hormone secretion, may be affected by cytokines, the effects of which are generally inhibitory. Due to the complexity of the cytokine network, it is difficult, however, to relate results of *in vitro* studies, which utilize chemically defined experimental conditions, to the *in vivo* situation which results from the complicated interplay of different cytokines. Experiments with single cytokines or even with a combination of cytokines may to some extent be misleading because they necessarily do not consider the milieu of other cytokines and hormones that may profoundly affect the action of an individual cytokine on cells.

### Effects of cytokines in the animal *in vivo*

An interesting animal model of experimental NTI is the injection of bacterial endotoxin (lipopolysaccharide, LPS) (67). Injection of a single, sublethal dose of LPS to mice resulted in systemic illness with hypothermia, induction of TNF- $\alpha$  and IL-6 after 1–2 h, decrease of hepatic 5'-DI after 4 h, decrease of TT<sub>4</sub> and TT<sub>3</sub> after 8 h, and of FT<sub>4</sub> and FT<sub>3</sub> after 24 h, with no change in TSH concentration (68). This temporal relationship might imply a cause-effect relationship between the increase in serum cytokine levels and the decrease in serum thyroid hormone concentrations. However, direct administration of TNF- $\alpha$  and IL-6 had no effect on thyroid function, whereas IFN- $\gamma$  caused a dose-dependent decrease in serum TT<sub>4</sub>, TT<sub>3</sub> and FT<sub>3</sub> concentrations, and IL-1 $\alpha$  caused systemic illness and a transient decrease in 5'-DI mRNA (68). This study thus suggested that LPS-induced ESS in mice is best explained, at least in an acute setting, by a combination of a direct thyroidal effect of IFN- $\gamma$  and an extrathyroidal inhibition of 5'-DI by IL-1 $\alpha$  (68). While the reduction in serum T<sub>3</sub> might be attributed to the diminished 5'-DI activity, the decrease in serum T<sub>4</sub> implies a direct effect at the thyroid level by LPS.

To evaluate further the role of IL-6, LPS was administered to IL-6 knock-out mice. While the decrease in serum T<sub>4</sub> concentration was similar in IL-6 knock-out and wild type mice, the decrease in serum T<sub>3</sub> concentration, as well as the reduction in 5'-DI activity, was smaller in IL-6 knock-out mice, suggesting a contribution of IL-6 to the pathogenesis of ESS (69). This would also be supported by the finding that immunoneutralization of

IL-6 did not prevent LPS-induced decreases in serum TT<sub>3</sub> and TT<sub>4</sub> concentrations, but it reduced the LPS-induced decrease in 5'-DI activity, while no effect was observed after immunoneutralization of IL-1, TNF- $\alpha$  and IFN- $\gamma$  (70). The acute subcutaneous administration of IL-6 (5  $\mu$ g) to rats was associated with a decrease in serum TT<sub>4</sub>, TT<sub>3</sub> and TSH concentrations (71, 72), while the T<sub>4</sub>/T<sub>3</sub> ratio decreased, suggesting that T<sub>4</sub> deiodination was not affected (72) (Table 2). IL-6 did not affect the pituitary TSH content, TSH- $\beta$  mRNA abundance, or hypothalamic TRH content (71). Changes in serum thyroid hormone concentrations could effectively be ascribed to IL-6, since they could be prevented by preincubation of IL-6 with its neutralizing antibody (72). The continuous intraperitoneal infusion of IL-6 (15  $\mu$ g/day for 7 consecutive days) in rats was associated with a transient decrease in serum TT<sub>4</sub> and TSH, although less than that caused by IL-1 (73) (Table 2). In the latter study, hypothalamic pro-TRH mRNA and pituitary TSH- $\beta$  mRNA were unaffected by IL-6, suggesting that the effects of IL-6 on TSH might not necessarily be associated with a decreased synthesis of thyrotropin (73). On the other hand, the observation that the intracerebroventricular administration of IL-6 to rats was followed by a decrease in serum TSH and an increase in serum adrenocorticotropin (ACTH) concentrations, while these changes could be reproduced in hemipituitaries only for ACTH, but not for TSH, suggested that the action of IL-6 on TSH might be exerted predominantly at the hypothalamic levels (74).

In rats, a single injection of IL-1 $\beta$  decreased serum TT<sub>3</sub>, TT<sub>4</sub> and TSH concentrations without affecting 5'-DI (75), whereas persistent changes in serum TT<sub>4</sub> and TT<sub>3</sub> levels were found during continuous infusion of the cytokine at doses not causing systemic disease (76) (Table 2). These changes, mostly related to decreased thyroid hormone binding to T<sub>4</sub>-binding prealbumin, were accompanied by a decrease in both basal and TRH-stimulated TSH levels, while rT<sub>3</sub> remained unchanged and 5'-DI was apparently unaffected (76). The effect of IL-1 on 5'-DI activity is not unequivocal, since an increase in liver 5'-DI activity was also reported in IL-1-treated mice (77). Likewise, a stimulation of 5'-DI activity was reported in the brain cortex of IL-1-treated rats (75). The continuous intraperitoneal infusion of IL-1 (4  $\mu$ g/day) in rats for 1, 2 or 7 days caused a decrease in serum TT<sub>4</sub>, TT<sub>3</sub>, FT<sub>4</sub> and TSH concentrations (73) (Table 2); changes in TSH were associated with a decrease in pro-TRH and TSH- $\beta$  mRNAs, indicating a site of action of the cytokine at both the hypothalamic and pituitary level (73). Interestingly, another study showed a prolonged effect of IL-1 $\alpha$  on mouse thyroid function; after a rapid decrease in serum TT<sub>4</sub> concentration following administration of 1 or 15  $\mu$ g IL-1 $\alpha$  daily for 7 consecutive days, serum TT<sub>4</sub> levels normalized after cytokine discontinuation, but decreased again 3 weeks after its withdrawal, in association with a decreased thyroid responsiveness to

**Table 2** Effects of cytokines on thyroid function in animals.

Cytokine	Animal species	Effects	Reference
IL-1 $\beta$	Rat*	Decreased TT <sub>4</sub> , TT <sub>3</sub> , TSH	75
	Rat**	Unchanged rT <sub>3</sub> , 5'-DI	75
TNF- $\alpha$	Mouse***	Decreased TT <sub>4</sub> , TT <sub>3</sub> , FT <sub>4</sub> , TSH	73, 76
		Decreased TT <sub>3</sub> , TT <sub>4</sub>	79
		Increased T <sub>3</sub> /T <sub>4</sub>	79
	Rat****	Unchanged rT <sub>3</sub>	79
		Decreased T <sub>3</sub> -T <sub>4</sub> response to TSH	79
		Decreased TT <sub>4</sub> , TT <sub>3</sub> , FT <sub>4</sub> , TSH	21
		Decreased hypothalamic TRH	21
		Decreased TSH glycosylation	21
		Decreased 5'-DI*****	80
		Rat*****	Decreased TT <sub>4</sub> , TT <sub>3</sub>
Unchanged basal and TRH-stimulated TSH	80		
Unchanged 5'-DI	77		
IL-6	Rat*	Decreased TT <sub>4</sub> , TT <sub>3</sub> , TSH	71, 72
	Rat**	Decreased TT <sub>4</sub> , TSH	73
IL-2	Dog	Decreased TT <sub>4</sub> , TT <sub>3</sub>	81

\* Acute experiment.

\*\* Continuous intraperitoneal infusion.

\*\*\* 3 days.

\*\*\*\* 1 day.

\*\*\*\*\* Increased after 3 days.

\*\*\*\*\* Continuous infusion.

TSH stimulation probably related to a decline in TSH-stimulated cAMP production (78).

The acute administration of TNF- $\alpha$  (1–100  $\mu$ g/day for 3 days) to mice was followed by a reduction in serum TT<sub>3</sub> and TT<sub>4</sub> concentrations, an increase in T<sub>3</sub>/T<sub>4</sub> ratio, and a decreased T<sub>3</sub> and T<sub>4</sub> response to TSH, while 5'-DI activity was unchanged (79) (Table 2). One-day treatment of rats with TNF- $\alpha$  caused a decrease in serum TT<sub>4</sub>, FT<sub>4</sub>, TT<sub>3</sub> and TSH concentrations, a reduction in hypothalamic TRH, an impaired glycosylation of TSH, a reduction in TSH- $\beta$  mRNA, and a reduction in T<sub>3</sub> and T<sub>4</sub> release (21) (Table 2). 5'-DI activity was reduced after 1 day of TNF- $\alpha$  treatment, but it increased after 3 days of treatment (21). Finally, the continuous infusion of TNF- $\alpha$  in rats in subpyrogenic and subanorectic doses caused a reduction in serum TT<sub>4</sub> and TT<sub>3</sub> concentrations without changes in basal and TRH-stimulated TSH concentrations, and apparently no changes in 5'-DI activity (80) (Table 2).

The continuous infusion of IL-2 in dogs (4 days per week for 2 weeks) caused a significant reduction in serum concentrations of TT<sub>4</sub> (25–50% of pretreatment values) and TT<sub>3</sub> (20–30% of pretreatment values), although cytokine administration was associated with severe systemic disease with diarrhea, vomiting and lethargy (81). Whether changes in thyroid hormone concentrations were a direct effect of IL-2 or were mediated by the induction of other cytokines by IL-2 was not established (81).

In summary, administration of cytokines to animals did not produce unequivocal results. Basically, a decrease in serum thyroid hormone concentrations

was often observed, sometimes in association with an inhibition of TSH secretion. Both central (hypothalamus–pituitary) and peripheral (thyroid, liver 5'-DI) actions of cytokines are likely. Differences were often reported between the acute and chronic effects of the same cytokine. It cannot be ruled out that, in addition to differences in animal species and experimental design, nutritional deficiencies may have contributed to the above changes and differences in the results of different studies. In this regard, it should be pointed out that control groups of pair-fed animals are lacking in many of the above studies. An inhibition of 5'-DI has not consistently been reported in animals. This might indicate that either the experimental design did not fully reproduce the situation in humans, or factors other than cytokines are indeed responsible for changes in thyroid function seen in ESS.

### Serum cytokine and cytokine receptor protein concentrations in patients with NTI

Serum T<sub>3</sub> concentration was lower in 29 nursing home residents who had detectable serum TNF- $\alpha$  concentration than in the 36 with undetectable TNF- $\alpha$ , while there was no differences in serum T<sub>3</sub> levels between patients with detectable or undetectable IL-1 $\alpha$  concentrations (82) (Table 3). No subject had an increase in serum rT<sub>3</sub> concentrations (82). Increased serum TNF- $\alpha$  and IL-6 (but not IL-1 $\beta$ ) concentrations were reported in 65 patients with African trypanosomiasis (sleeping sickness) (83); a negative correlation between TNF- $\alpha$

**Table 3** Serum cytokine and cytokine receptor protein (Rp) levels in NTI.

Cytokine	Serum levels	Reference
TNF- $\alpha$	Often increased Usually normal	82, 83 84
IL-6	Increased	83, 85–93
IL-8	Undetectable	94
IL-10	Undetectable	94
IFN- $\gamma$	Undetectable	94
sTNF- $\alpha$ Rp55	Increased	95
sTNF- $\alpha$ Rp 75	Increased	95
sIL-2 receptor	Increased	95
IL-1 receptor antagonist	Normal/increased	95

Included in the different series were: nursing home residents (82), patients with African trypanosomiasis (83), children with acute respiratory infections (88), brain-dead patients (89), patients with recurrent breast cancer (90), patients submitted to surgery (91) or cardiopulmonary bypass (92), patients with chronic liver, renal, cardiac and gastrointestinal disorders or diabetes mellitus (84, 85–87, 93, 94).

and FT<sub>3</sub> values was also found (83). It should be noted that patients in this study had an increase in the mean serum TSH concentration compared with control subjects, suggesting that changes in thyroid hormone levels can be more likely ascribed to primary thyroid failure (83). Serum rT<sub>3</sub> concentration were normal in sleeping sickness (83). The relevance of TNF- $\alpha$  changes in NTI patients was questioned, based on the observation that serum concentration of the cytokine was elevated in only 1 of 13 NTI patients (84); no relationship was found between TNF- $\alpha$  and thyroid hormone concentrations, with the exception of a slight correlation with FT<sub>4</sub> values, possibly related to a TNF- $\alpha$ -induced increase in FFA levels (84) (Table 3).

An increase in serum IL-6 concentrations was reported in NTI patients, especially those with low T<sub>3</sub> concentration (83, 85–87) (Table 3). Serum IL-6 levels were negatively correlated with serum FT<sub>3</sub> and positively correlated with serum rT<sub>3</sub> concentrations (86). Increased serum IL-6 concentrations were also found in the majority of 59 children with acute respiratory infections (88), in brain-dead patients (89) and in women with breast cancer (90), with an inverse relationship with serum T<sub>3</sub> values and the T<sub>3</sub>/T<sub>4</sub> ratio (88–90). Interestingly, TNF- $\alpha$  and IL-1 concentrations were generally normal (88, 89). A temporal relationship between IL-6 and thyroid hormone variations was observed in the post-surgery period, since the increase in serum IL-6 concentration occurred early and preceded the decrease in serum T<sub>3</sub> concentration both in adults (91) and in children undergoing cardiopulmonary bypass (92). A recent evaluation of 270 in-patients with NTI, while confirming changes in IL-6 levels and their relationship with variations in serum thyroid hormone concentrations, showed that the IL-6 increase was modest in patients with acute or chronic renal disorders, in spite of the concomitant decrease in

serum thyroid hormone concentrations, suggesting that, at least in this form of NTI, IL-6 is not the only or most important causative factor responsible for alterations in thyroid hormone metabolism (93).

Other cytokines, including IL-8, IL-10 and IFN- $\gamma$ , were undetectable and not related to changes in serum thyroid hormone concentration of NTI patients (94) (Table 3).

An increase in serum soluble cytokine receptor proteins (Rp) was reported in NTI patients, in particular soluble TNF- $\alpha$  receptor protein 55 (sTNF- $\alpha$  Rp55), soluble TNF- $\alpha$  receptor protein 75 (sTNF- $\alpha$  Rp75) and soluble IL-2 receptor (95) (Table 3). Although all these receptor proteins, as well as IL-1 receptor antagonist, showed a negative correlation with serum T<sub>3</sub> values, stepwise multiple regression indicated that only sTNF- $\alpha$  Rp75 and IL-6 were independent determinants of T<sub>3</sub>, accounting for 35 and 14%, respectively, of T<sub>3</sub> changes in NTI (95). Since IL-6 and TNF- $\alpha$  Rp are considered as anti-inflammatory proteins, this relationship might be regarded as a mechanism by which the body counteracts systemic disease.

In summary, increased concentrations of IL-6 and, to a lesser extent, TNF- $\alpha$  are often found in NTI patients. The increase in IL-6 levels might reflect stimulation of its synthesis by other cytokines such as IL-1 and TNF- $\alpha$  that might be undetectable in the circulation because they are only transiently increased and act mainly via autocrine and paracrine mechanisms. Thus, in view of the mentioned interaction of cytokines with each other and with hormones, determination of serum cytokine concentration may unravel only part of the story. The increased levels of cytokine receptor proteins probably reflect the activation of the cytokine network during the acute phase reaction occurring during acute and chronic disorders (7). All the above described studies do not answer the question of whether the increase in cytokine circulating levels is the cause of changes in thyroid hormone concentration and metabolism or reflects a concomitant (and independent) alteration due to systemic disease.

### Effects of cytokine administration in humans

Only a few studies so far have evaluated the relationship of administration of cytokines to humans with changes in thyroid economy. It should be pointed out that all these studies carry the limitation of being open, unrandomized and uncontrolled.

Infusion of TNF- $\alpha$  (50  $\mu$ g/m<sup>2</sup>) for 5.5 h in six healthy volunteers was followed, throughout the 10.5 h of follow-up, by a significant decrease in serum TT<sub>3</sub> and TSH concentrations and a significant increase in rT<sub>3</sub> values, while FT<sub>4</sub> showed a transient increase, synchronous with and possibly related to the increase in FFA levels (96) (Table 4).

**Table 4** Effects of cytokine administration in humans.

Cytokine	Dose	Subjects	Effects	Reference
TNF- $\alpha$	50 $\mu\text{g}/\text{m}^2$	Six healthy volunteers	Decreased TT <sub>3</sub> , TSH Increased rT <sub>3</sub> , FFA	96
IFN- $\alpha$ 2b	$5 \times 10^6$ U/m <sup>2</sup>	Eight healthy volunteers	Decreased TT <sub>3</sub> , TSH Increased rT <sub>3</sub> , IL-6 Unchanged TT <sub>4</sub>	100
IL-6	150 $\mu\text{g}$ i.v. (4 h)	Eight renal cancer patients	Decreased TT <sub>3</sub> , TSH Increased rT <sub>3</sub> Unchanged TT <sub>4</sub> , FT <sub>4</sub>	101
	150 $\mu\text{g}$ s.c. (42 days)	Eight renal cancer patients	Early changes, subsequent normalization	101
IL-2*	$3 \times 10^6$ U/m <sup>2</sup>	Four hepatocarcinoma patients	Decreased TT <sub>4</sub> , FT <sub>4</sub> , TT <sub>3</sub>	108

\* Associated with lymphokine-activated killer cells.

IFN- $\alpha$  long-term administration for chronic hepatitis and malignant disorders can be responsible for thyroid dysfunction, both hyper- (97) and hypothyroidism (98), usually accompanied by a rise in circulating thyroid autoantibodies. Withdrawal of the drug was usually associated with normalization of thyroid function (99) (Table 4). Subcutaneous injection of IFN- $\alpha$  2b ( $5 \times 10^6$  U/m<sup>2</sup>) in eight healthy volunteers significantly reduced, within a few hours, serum TSH and TT<sub>3</sub>, increased rT<sub>3</sub> and did not affect T<sub>4</sub> concentrations (100) (Table 4). The effect of IFN- $\alpha$  was slightly slower than that reported for TNF- $\alpha$ , possibly due to the different route of cytokine administration (intravenous for TNF- $\alpha$ , subcutaneous for IFN- $\alpha$ ). Further to IFN- $\alpha$  administration, IL-6 increased, while IL-1 and TNF- $\alpha$  levels did not change, suggesting that the effects of IFN- $\alpha$  might be mediated, at least partially, by IL-6 (100).

The effects of IL-6 were evaluated after either acute or chronic administration in patients with renal cancer (101). The acute (4 h) intravenous administration of 150  $\mu\text{g}$  IL-6 to eight patients was followed by a significant decrease in serum TT<sub>3</sub> and TSH concentrations, with an increase in serum rT<sub>3</sub> levels and no change in serum TT<sub>4</sub> and FT<sub>4</sub> concentrations (101) (Table 4). On the other hand, the chronic (42 days) subcutaneous administration of 150  $\mu\text{g}$  daily of IL-6 to eight patients was associated with early and transient changes in thyroid parameters similar to the acute experiment, followed by normalization of thyroid hormone and TSH levels after a few weeks and even before discontinuation of the drug (101) (Table 4). This might suggest that, while IL-6 might contribute to the development of ESS, factors other than IL-6 could be involved in the persistence of changes in thyroid parameters during chronic illness.

Due to the effects that LPS has in the animal, experiments on the acute effects of this substance were carried out also in humans. The intravenous administration of LPS (20 U/kg) to 18 healthy volunteers was followed by a rapid decrease in serum TT<sub>4</sub>, FT<sub>4</sub>,

TT<sub>3</sub> and TSH and a rise in rT<sub>3</sub> concentrations (102). Interestingly, these changes could not be prevented by coinfusion of IL-1 receptor antagonist, apparently administered at a high molar excess, suggesting that IL-1 does not play a pivotal role in the pathogenesis of changes in thyroid economy encountered in mild endotoxemia (102). On the other hand, TNF- $\alpha$  and IL-6 are readily detected in the bloodstream after administration of low-dose endotoxin to humans (103, 104) and might be responsible for ESS changes.

Although treatment with IL-2 may cause hyper- or, more frequently, hypothyroidism (105–107), changes typical of ESS, such as a reduction in serum TT<sub>3</sub>, TT<sub>4</sub> and FT<sub>4</sub>, with no changes in serum TSH concentrations, were also reported in four of eight patients with hepatocellular carcinoma after 16 courses of treatment with IL-2 (and lymphokine-activated killer cells) (108) (Table 4). The relative contribution of lymphokine-activated killer cells or disease itself is difficult to establish, but no abnormalities of thyroid function were seen in nine cancer patients receiving no active treatment (108).

In summary, the limited studies available demonstrated that TNF- $\alpha$ , IFN- $\alpha$ , IL-6 and endotoxin administration is followed by rapid changes in thyroid parameters similar to those found in ESS. It should, however, be pointed out that most investigations were carried out acutely. The effects of acute cytokine administration might be related to the systemic (flu-like) illness caused by the cytokine rather than to the effect of the cytokine itself. On the other hand, the rapidity with which changes in thyroid hormone and TSH concentrations occur after cytokine administration might imply that these changes do not merely constitute an adaptation to the long-standing catabolic state or caloric deprivation of NTI. Data on chronic administration of cytokines are scanty. Chronic administration of IL-6 did not provide clear results (101), whereas chronic administration of IL-2 was associated with changes typical of ESS (108). Admittedly, controlled studies are warranted, because the latter results might simply reflect

progression of neoplastic disease. Experiments in which the effects of cytokines are blocked by cytokine antagonists are also needed, because this approach might help to identify the specific role of individual cytokines. In humans, the only available study showed that IL-1 receptor antagonist did not block endotoxin-induced changes in thyroid parameters (102), while in the rat IL-6-induced thyroid hormone changes could be prevented by IL-6 immunoneutralization (72).

### Are cytokines responsible for ESS?

The experimental and clinical data presented above underscore the concept that there are elements both in favor of and against a relevant role of cytokines in the pathogenesis of ESS.

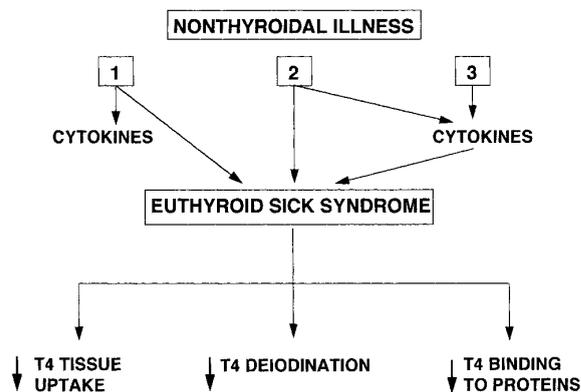
#### Elements against

- (1) Studies *in vivo* either in animals or in humans caused changes in thyroid function similar to those of ESS, but they often employed pharmacological doses of cytokines that might cause systemic disease responsible for thyroid function variations.
- (2) Most studies in humans were carried out acutely, while controlled studies on chronic effects of cytokine administration are lacking.
- (3) Serum concentrations of several cytokines are often normal in NTI.
- (4) Serum concentrations of cytokines do not necessarily reflect paracrine or autocrine effects.
- (5) 5'-DI activity is not always decreased by cytokines, both in *in vivo* and *in vitro* studies.

#### Elements in favor

- (1) Increased concentrations of cytokines, especially IL-6, are often found in NTI patients and correlate with changes in thyroid hormone concentrations.
- (2) Cytokines profoundly affect *in vitro* thyroid function, either directly or indirectly via regulation of TSH synthesis and secretion.
- (3) The administration of cytokines *in vivo* mimic, at least acutely, changes of thyroid function tests occurring in NTI, and the induced changes in thyroid function tests occur so quickly that it is difficult to attribute them to cytokine-induced systemic disease.
- (4) Neutralization of IL-6 effects by anti-IL-6 antibody was reported in animals.

Thus, while it seems certain that NTI is associated with an increased production and release of cytokines, the degree of cytokines involvement and their specific role in the pathogenesis of ESS remain to be clarified. One possibility is that NTI *per se* causes an increase in cytokine production and the latter is the only factor responsible for changes in ESS (Fig. 2). A second possibility is that illness, through mechanisms



**Figure 2** Possible relationship between cytokines and ESS. In model 1, nonthyroidal illness is, at the same time, responsible for ESS and the increased production of cytokines, but these are not involved in the pathogenesis of ESS. In model 3, nonthyroidal illness causes an increased production of cytokines, which are solely responsible for ESS. In model 2, cytokines contribute to the pathogenesis of ESS, but other mechanisms, cytokine-unrelated and disease-related, are also involved. Adapted and expanded from Wiersinga & Boelen (7).

incompletely understood, produces both an increase in cytokine levels and changes in thyroid function tests, the latter being totally independent of cytokine variations (Fig. 2). The third and more likely model relates changes in thyroid parameters to both a direct effect of disease and the illness-related increase in cytokine production and release (Fig. 2). This model best fits with available data indicating that, in addition to the above discussed *in vitro* and *in vivo* effects of cytokines, other substances, such as CMPE, indoxyl-sulfate, bilirubin and FFA, which reduce thyroid hormone transport into cells and thereby decrease peripheral T<sub>3</sub> production, probably participate in the pathogenesis of ESS (7).

### Anticytokine strategy for ESS?

If cytokines are involved in the pathogenesis of ESS, then control of their enhanced production and action might deserve consideration. Regulation of cytokine activity might be achieved through different mechanisms. Soluble cytokine receptors are highly selective inhibitors that bind cytokines and prevent their subsequent binding to their receptor: receptor antagonists and mutated cytokines bind to cytokine receptors, thus preventing subsequent receptor-induced signal transduction; antibodies to cytokines neutralize and thus antagonize cytokines; nonpeptidic antagonists (e.g. isothiazolone A) probably interfere with cytokine synthesis, receptor binding or signal transduction (109). Information on the effectiveness of anticytokine therapy is currently rather limited, and support from properly carried out controlled studies is warranted. With this limitation in mind, can an anticytokine strategy be envisaged for correction of ESS? First, although, as discussed above, growing evidence supports

a role for cytokines in ESS, this remains to be definitely proven and defined. Secondly, it is unclear which cytokine should be blocked. Cytokines are related to each other in a very complex network, and regulate positively or negatively the expression of other cytokines; it is, therefore, difficult to imagine how to interrupt this interplay and cascade of events. In addition, it would be difficult to determine doses of the antagonist to be used and the length of treatment. Cost/benefit considerations should also be made, especially because, and this is the most important point, it remains to be ascertained whether changes in thyroid economy occurring in NTI really need to be corrected. A leading textbook states that '... change in thyroid function may serve the purpose of conserving energy by a diminished provision of biologically active thyroid hormone in order to compensate for the increased metabolic demands imposed by the disease ...' (110). In agreement with this concept, the use of thyroid hormones did not beneficially affect the course of NTI (111). The use of anticytokine strategies, although aimed at the purported mechanisms of ESS and not at their consequences in terms of thyroid economy, would probably not achieve better results.

## Acknowledgements

We wish to thank Professor Aldo Pinchera for his continuous encouragement and advice. This work was supported in part by grants from the National Research Council (CNR, Rome, Italy) and by University of Pisa (Fondi di Ateneo).

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Received 13 November 1997

Accepted 5 February 1998