Neutralization of TNF does not influence endotoxin-induced changes in thyroid hormone metabolism in humans

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Van Der Poll, Tom, Erik Endert, Susette M. Coyle, Jan M. Agosti, and Stephen F. Lowry. Neutralization of TNF does not influence endotoxin-induced changes in thyroid hormone metabolism in humans. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R357–R362, 1999.—To determine the role of tumor necrosis factor (TNF) in endotoxin-induced changes in plasma thyroid hormone and thyroid-stimulating hormone (TSH) concentrations, 24 healthy postabsorptive humans were studied on a control study day (n = 6), after infusion of a recombinant TNF receptor IgG fusion protein (TNFR:Fc; 6 mg/m²; n = 6) after intravenous injection of endotoxin (2 ng/kg; n = 6), or after administration of endotoxin with TNFR:Fc (n = 6). Administration of TNFR:Fc alone did not affect thyroid hormone or TSH levels when compared with the control day. Endotoxin induced a transient rise in plasma TNF activity (1.5 h: 219 ± 42 pg/ml), which was completely prevented by TNFR:Fc (P < 0.05). After endotoxin administration, plasma L-thyroxine (T₄), free T₄, T₃ triiodothyronine, and TSH were lower and 3,3',5',triiodothyronine (T₃), and TSH were lower and 3,3',5'-triiodothyronine was higher than on the control day (all P < 0.05). Coinfusion of TNFR:Fc with endotoxin did not influence these endotoxin-induced changes. Our results suggest that endogenous TNF does not play an important role in the alterations in plasma thyroid hormone and TSH concentrations induced by mild endotoxaemia in healthy humans.

Lipopolysaccharide; cytokines; thyrotropin

THE EUTHYROID SICK SYNDROME is characterized by changes in thyroid hormone metabolism in patients with systemic nonthyroidal illness (NTI) who are clinically euthyroid (8, 34). Characteristically this syndrome involves a decrease in the serum concentrations of 3,3',5'-triiodothyronine (T₃) and an increase in 3,3',5'-triiodothyronine (T₄), L-thyroxine (T₄) and thyroid-stimulating hormone (TSH) levels usually remain normal but can be decreased in severe NTI (8, 21, 35). Many systemic NTI are associated with enhanced production of proinflammatory cytokines, among which interleukin (IL)-1 and tumor necrosis factor (TNF) are the most potent (5, 39). Increased activity of these mediators has been implicated in the development of altered thyroid hormone metabolism in NTI.

Recently, we established a human model of the euthyroid sick syndrome (31). Intravenous administration of low-dose endotoxin (lipopolysaccharide, LPS) to healthy subjects reproduced a number of changes in the plasma concentrations of thyroid hormones and TSH commonly seen in NTI, including reduced T₄, T₃, and TSH concentrations, and increased rT₃ levels. We used this model to determine the role of IL-1 in LPS-induced changes in thyroid hormone metabolism by blocking endogenous IL-1 activity through treatment with recombinant IL-1 receptor antagonist. It was found that IL-1 receptor blockade did not affect the alterations in thyroid hormone and TSH levels in low-grade endotoxia, suggesting that IL-1 does not play a significant role in this LPS effect (33).

TNF can influence thyroid hormone metabolism at multiple levels (reviewed in Ref. 7). In rats, a single intravenous injection of TNF resulted in a decrease in TSH, T₄, and T₃ levels within 4 h (13). In healthy humans, intravenous administration of recombinant TNF resulted in decreases in T₃ and TSH plasma concentrations and an increase in plasma rT₃ levels (32). Endogenous TNF has been implicated as an important mediator of LPS-induced host responses (26, 29, 30). Therefore, we considered it of interest to determine the role of endogenous TNF activity in the changes in plasma thyroid hormone and TSH concentrations elicited by intravenous LPS in humans. For this purpose we performed a placebo-controlled study in healthy humans exposed to a single intravenous dose of LPS in conjunction with a (TNF neutralizing) recombinant dimeric TNF receptor IgG fusion protein (TNFR:Fc).

MATERIALS AND METHODS

Study design. The present study was performed simultaneously with an investigation examining the effect of TNF neutralization on LPS-induced clinical, leukocyte, and cytokine responses, of which the results have been reported in detail (27, 28). Twenty-four adult male subjects, aged 27 ± 1 (mean ± SE) yr, were admitted to the Adult Clinical Research Center after documentation of good health by history, physical examination, and hematologic and biochemical screening. The study was approved by the Institutional Review Board, and written informed consent was obtained from all subjects before enrollment in the study. Subjects were allowed no intake of food from 10:00 PM on the night before the study until 12 h after endotoxin or placebo administration (9:00 PM). During this time they had free access to water. Twelve subjects received an intravenous injection with LPS (National Reference Endotoxin, Escherichia coli 0113 [lot EC-5], generously provided by Dr. H. D. Hochstein, the Bureau of Biologics, Food and Drug Administration, Bethesda, MD) at a dose of 2 ng/kg body wt at 9:00 AM. These 12 subjects were
randomized to also receive a 30-min intravenous infusion (starting at 8:30 AM) with either a recombinant dimeric TNF receptor (TNFR:Fc; Immunex, Seattle, WA) at a dose of 6 mg/m² (n = 6) or vehicle (n = 6). The remaining 12 subjects were not injected with endotoxin, but were randomized to receive a 30-min intravenous infusion of TNFR:Fc (n = 6) or vehicle (n = 6). TNFR:Fc was manufactured by fusing two identical extracellular portions of the type II (p75) TNF receptor with the Fc domain of IgG1 (9). The resulting dimeric TNF receptor binds TNF with a 50-fold greater affinity (inhibition constant = 10⁻¹⁰ M⁻¹) than monomeric receptor (9). TNFR:Fc was reconstituted with 1 ml of sterile water for injection from a lyophilized powder containing (in mg) 10 TNFR:Fc, 1.2 Tris, 10 sucrose, and 40 mannitol. The final dose of TNFR:Fc (or vehicle) was diluted in 100 ml of isotonic saline before infusion. Two hours before the administration of LPS (or saline) a radial arterial catheter was placed in all subjects to continuously monitor heart rate and blood pressure (Datascope model 2000A, Datascope, Paramus, NJ) and for blood sampling. Arterial blood was obtained at 8:30 AM (i.e., directly before the start of the infusion with TNFR:Fc or vehicle, t = 0 h); directly before the injection of LPS or saline (t = 0 h); and 1, 2, 3, 4, 5, 6, 8, and 12 h thereafter.

Assays. The following assays were used: TSH (Delfia, Wallac, Turku, Finland), T₄, T₃, and rT₃ by RIAs (36), and free T₄ (Delfia). Coefficients of variation of these assays (intra- and interassay, respectively) are TSH (2–3% and 3–5%), T₄ (3–6% and 4–7%), T₃ (2–6% and 3–6%), rT₃ (2–6% and 3–6%), and free T₄ (4–7% and 7–9%).

Statistical analysis. Comparisons between different groups were performed exactly as described previously (33). All values are given as means ± SE. Primary data were analyzed by repeated-measures analysis of variance (interaction between time and treatment). All listed P values are derived from this analysis (of primary data). For reasons of clarity, Figs. 1–3 show data as percentage deviation from baseline. These values were analyzed by repeated-measures analysis of variance (interaction between time and treatment) after logarithmic transformation, which revealed similar results in terms of differences between treatment groups as found with the analysis of primary data. P < 0.05 was considered to represent a significant difference.

RESULTS

Plasma TNF activity and clinical responses. Details of the in vivo neutralizing capacity of TNFR:Fc at the dose administered have been reported elsewhere (28). Infusion of TNFR:Fc effectively neutralized LPS-induced TNF activity. In subjects injected with LPS only, plasma TNF activity peaked after 1.5 h (219 ± 42 pg/ml; P < 0.05 versus time), whereas in subjects infused with LPS and TNFR:Fc TNF activity remained undetectable (P < 0.05 vs. LPS only). Furthermore, addition of 10 ng/ml recombinant TNF to plasma samples obtained from TNFR:Fc-treated subjects at 1.5 or 24 h after injection of LPS did not result in detectable TNF activity, indicating that the amount of TNFR:Fc administered offered an excess of TNF neutralizing capacity. LPS induced a febrile response that was significantly blunted by TNFR:Fc (28); peak temperatures were 38.2 ± 0.1°C (LPS only) and 37.7 ± 0.1°C (LPS with TNFR:Fc; P < 0.05). TNFR:Fc did not significantly influence flu-like symptoms induced by LPS, including chills, headache, and nausea. In the groups that were not injected with LPS, TNF activity remained undetectable in plasma and no clinical signs or symptoms were registered.

Effect of TNFR:Fc only. Infusion of TNFR:Fc did not influence plasma thyroid hormone or TSH concentrations when compared with the sham day (Figs. 1–3). T₄ and free T₄. Figure 1 shows plasma concentrations of T₄ and free T₄. After administration of LPS only, T₄ levels showed inconsistent alterations, varying between 83 ± 2 and 87 ± 4 nmol/l, while they modestly increased on the sham study day (P < 0.05 for the difference between LPS only and sham). Infusion of TNFR:Fc before LPS injection did not influence T₄ levels (P = 0.31 for the difference with LPS only). However, T₄ concentrations in the LPS+TNFR:Fc group were different from those measured at the control day (P < 0.05). Administration of LPS only resulted in a decrease in free T₄ levels from 13.1 ± 0.8 to 11.9 ± 0.8 pmol/l after 12 h (percentage deviation from baseline: −8.7 ± 3.0; P < 0.05 for the difference with sham). Free T₄ concentrations decreased similarly in both LPS-treated groups (P = 0.80 for the difference between LPS only and LPS+TNFR:Fc).

Fig. 1. Mean (± SE) plasma concentrations of l-thyroxine (T₄; A) and free T₄ (B) expressed as percentage deviation from baseline. Data are shown for each treatment group: sham (○), recombinant tumor necrosis factor (TNF) receptor IgG fusion protein (TNFR:Fc) alone (□), lipopolysaccharide (LPS) alone (●), and both LPS and TNFR:Fc (▲). In comparison with the sham day, T₄ and free T₄ levels were lower after injection of LPS (P < 0.05). These LPS effects were not influenced by coinfusion of TNFR:Fc.
Hence, when compared with the sham day, administration of LPS was associated with lower T4 and free T4 concentrations, which was not influenced by the concurrent infusion of TNFR:Fc.

T3 and rT3. Figure 2 shows plasma concentrations of T3 and rT3. Injection of LPS only was associated with a decrease in T3 concentrations when compared with the sham day (from 1.61 ± 0.04 to 1.33 ± 0.07 nmol/l after 12 h; percentage deviation from baseline: −17.4 ± 4.5; P < 0.05 for the difference with sham). Infusion of LPS with TNFR:Fc also resulted in a decrease in T3 levels when compared with the sham day (P < 0.05 for the difference with sham). The changes in T3 levels found after injection of LPS only and after administration of LPS with TNFR:Fc were similar (P = 0.97). LPS induced an increase in rT3 levels when compared with the sham day (from 0.17 ± 0.01 to 0.30 ± 0.02 nmol/l after 12 h; percentage deviation from baseline: +75.1 ± 6.1; P < 0.05 for the difference with sham). After infusion of LPS with TNFR:Fc rT3 concentrations also increased (P < 0.05 for the difference with sham). The alterations in rT3 induced by LPS only were similar to those elicited by LPS with TNFR:Fc (P = 0.63).

Thus compared with the sham study day, LPS administration resulted in a decrease in T3 levels and an increase in rT3 levels, changes that were not affected by infusion of TNFR:Fc.

TSH. Figure 3 shows plasma concentrations of TSH. LPS administration was associated with a decrease in TSH levels when compared with the sham day (from 1.92 ± 0.21 to 0.78 ± 0.09 mU/l after 8 h; percentage deviation from baseline: −59.5 ± 2.0; P < 0.05 for the difference with sham). Infusion of LPS with TNFR:Fc also resulted in a decrease in TSH levels (P < 0.05 for the difference with sham). The alterations in TSH levels detected after LPS only and after LPS with TNFR:Fc were similar (P = 0.87).

Hence compared with the sham study day, LPS induced a decrease in TSH concentrations that was not affected by administration of TNFR:Fc.

DISCUSSION

Administration of LPS to healthy subjects is a widely used model to study the human response to gram-negative infection (31). In an earlier study we demonstrated that intravenous injection of low-dose LPS reproduces a number of changes in thyroid hormone metabolism commonly seen in NTI (33). Because TNF can induce a euthyroid sick syndrome in various species, including humans (7, 11, 13, 32), we sought to determine the role of this proinflammatory cytokine in LPS-induced changes in thyroid hormone and TSH concentrations. It was found that neutralization of endogenous TNF activity by infusion of TNFR:Fc does not influence the alterations in thyroid hormone and TSH levels in low-grade endotoxemia. Thus our data suggest that TNF does not play a significant role in this human model of the euthyroid sick syndrome.

Thyroid hormone metabolism can be influenced by starvation and circadian variation (4, 23, 32), which may explain at least part of the changes observed during the control study periods. It is therefore important to determine changes in plasma thyroid hormone and TSH concentrations in a controlled setting. Although the observed changes after endotoxin adminis-
tation were relatively small and sometimes in the same direction as those in the control study periods, we consider the registered alterations in the plasma concentrations of thyroid hormones and TSH to be a reflection of an endotoxin effect, because comparisons were made between study days that only differed with respect to endotoxin administration.

In vitro studies have indicated that TNF can affect thyroid function directly. Normal human thyrocytes have been shown to possess specific receptors for TNF (2). Rat FRTL-5 thyroid cells also express TNF receptors, the number of which are regulated by TSH (12). TNF can inhibit basal and TSH-stimulated uptake of thyroid hormones by FRTL-5 cells, slow the recovery of trapping after exposure of cells to TSH, and augment the loss of the 

\[ {\text{trapping function after deprivation of cells of TSH}} \]

(14). In addition, TNF can decrease TSH-induced 5'-deiodinase activity in FRTL-5 cells, by which it may interfere with the production of T3 by the thyroid gland (10, 16, 17, 25). In cultures of human thyrocytes, TNF has been found to impair TSH-induced incorporation of Na+-K+-ATPase, which provides the Na+-electrochemical gradient driving iodide uptake (17, 18, 20, 22). Hence TNF suppresses thyroid function in vitro by inhibiting both thyroid hormone synthesis and secretion. TNF may also directly affect pituitary cells by inhibiting thyrotropin-releasing hormone (TRH)-stimulated TSH secretion (15). Interestingly, human thyroid epithelial cells are able to produce TNF in vivo, indicating that this cytokine can have autocrine effects on thyroid function (37).

In rodents in vivo, the systemic administration of TNF reproduces the major features of the euthyroid sick syndrome (11, 13). In rats, decreases in serum TSH and T3 and T4 concentrations were associated with a reduced amount of TRH in the hypothalamus, decreased TSH mRNA in the pituitary, impaired uptake, reduced responsiveness at the level of the thyroid, and decreased 5'-deiodinase activity in the liver (13), indicating that TNF can influence the hypothalamic-pituitary-thyroid axis at multiple levels. Similarly, in healthy humans a bolus intravenous injection of TNF induced decreased serum levels of TSH and T3 and increased serum concentrations of rT3 (32).

To our knowledge, our study is the first to establish the role of endogenous TNF in a model of the euthyroid sick syndrome. Infusion of TNFR:Fc at a dose of 6 mg/m² effectively neutralized TNF activity produced in response to intravenous LPS. Recently, TNFR:Fc given at higher doses (10 and 60 mg/m²) was found to have a paradoxical effect on cytokine and stress hormone release during human endotoxemia, i.e., as the dose of TNFR:Fc increased, the degree of inhibition decreased (24). Because the lower TNFR:Fc dose used in the earlier volunteer study still provided a large excess of TNF neutralizing capacity (24), we chose to administer TNFR:Fc at 6 mg/m². In both our and the previous investigation, TNF activity remained neutralized up to 24 h after LPS injection, thereby ruling out the occurrence of delayed and prolonged release of TNF activity from TNF-TNF-Fc complexes, as has been reported in a model of murine sepsis (6). Furthermore, the administration of TNFR:Fc only (i.e., without LPS) was not associated with any inflammatory response, including changes in thyroid hormone and TSH concentrations (Ref. 28 and the present study).

We have shown previously that, similar to TNF, endogenous IL-1 activity does not play a role of importance in the alterations in thyroid hormone and TSH concentrations during human endotoxemia (33). Which mediators do then cause these changes? A potential secondary mediator is IL-6, which can be readily detected in the circulation after administration of a low dose of LPS to normal humans (28, 31). Although infusion of TNFR:Fc significantly reduced LPS-induced IL-6 secretion, IL-6 release was not completely prevented (28). Intravenous infusion of IL-6 to renal cancer patients caused a decrease in the plasma concentrations of T3 and TSH and an increase in rT3 levels (23). Furthermore, in IL-6 gene-deficient mice the low-T3 syndrome, induced by either endotoxin, Listeria monocytogenes infection, or turpentine administration, was less marked than in normal wild-type mice (1). Thus studies in humans in whom endogenously produced IL-6 is neutralized completely are required to determine its role in the altered thyroid hormone metabolism in human NTI. Another cytokine that can induce a temporary euthyroid sick syndrome in humans is interferon-α (3). It is at present unclear, however, if and to what extent interferon-α is produced during endotoxemia.

In conclusion, intravenous injection of LPS in healthy humans induced a transient increase in plasma TSH activity in association with alterations in plasma thyroid hormone and TSH concentrations that resemble the euthyroid sick syndrome. Although TNF effects on hypothalamic-pituitary-thyroid axis function have been documented extensively, neutralization of endogenous TNF activity had no influence on LPS-induced changes in thyroid hormone metabolism. Further studies are warranted to study the role of endogenous TNF in other experimental models of NTI, for example by using TNF gene-deficient mice. Nonetheless, the present data argue against a role of endogenous TNF in the euthyroid sick syndrome in at least some forms of NTI.

Perspectives

A wide variety of diseases can be associated with changes in the plasma concentrations of thyroid hormones and TSH. Because patients with these diseases clinically are euthyroid, this syndrome is generally referred to as the euthyroid sick syndrome. TNF-α is a proinflammatory protein of which the production is increased during many different diseases. In vitro and animal studies have revealed that TNF-α can influence thyroid function at multiple levels. In addition, TNF-α injection in humans has been found to reproduce the major characteristics of the euthyroid sick syndrome.
In the present investigation, we used a human model of systemic inflammation elicited by intravenous injection of endotoxin to induce a euthyroid sick-like syndrome in healthy subjects. Endotoxin not only induced changes in plasma thyroid hormone levels, but also a transient rise in TNF-α concentrations. Neutralization of this endogenous TNF-α did not influence the altered thyroid hormone metabolism during endotoxemia. Therefore, it appears that although TNF-α can reproduce changes in thyroid hormone and TSH levels found in “disease,” endogenous TNF-α is not required for these changes. Hence, interference with TNF-α activity in clinical diseases will not necessarily modify the euthyroid sick syndrome that can be associated with such diseases.

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REFERENCES


