Autoinduction of tumor necrosis factor- α in FRTL-5 rat thyroid cells

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Abstract

Tumor necrosis factor- α (TNF α) may play a role in the development of autoimmune thyroiditis such as Hashimoto's thyroiditis. In the present study, we examined whether TNF α induced its own expression in FRTL-5 rat thyroid cells. Lipopolysaccharide (LPS) markedly increased TNF α mRNA levels in FRTL-5 cells as assessed by semiquantitative RT-PCR. In addition, LPS-stimulated cells released TNF α protein into the culture medium. Similarly, TNF α induced its own gene and protein expression in FRTL-5 cells as assessed by RT-PCR and metabolic labeling and immunoprecipitation of TNF α . The autoinduction of TNF α gene was also observed in TNF α -stimulated human thyroid epithelial cells. TNF α induction was specific to LPS and TNF α since interferon- γ or amiodarone failed to increase TNF α

mRNA levels in FRTL-5 cells. Human TNF α induced rat TNF α gene expression, indicating that type 1 TNF receptor (TNF-R) is involved in the autoinduction. TNF α did not increase either type 1 or type 2 TNF-R mRNA levels, suggesting that upregulation of TNF receptors is not involved in the autoinduction of TNF α . Although the biological significance of autoinduction of TNF α remains unclear, our results suggest that thyroid epithelial cells may participate in the development of autoimmune thyroiditis through production of TNF α . Furthermore, inhibition of TNF α production in the thyroid may represent a novel approach to mitigating inflammation in autoimmune thyroiditis.

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Introduction

Tumor necrosis factor- α (TNF α) is a pleiotropic cytokine that acts as a central regulator of inflammation and immunity. This cytokine is detectable in thyroid tissues obtained from patients with Hashimoto's thyroiditis (Ajjan *et al.* 1996, Aust *et al.* 1996). In accordance, we and others have demonstrated enhanced TNF α gene expression in the inflamed thyroid of BioBreeding/Worcester (BB/W) rats (Mori *et al.* 1998, Bluher *et al.* 1999). Furthermore, TNF α augments interferon- γ (IFN γ)-induced class II major histocompatibility complex (MHC) antigen expression (Weetman & Rees 1988, Zakarija *et al.* 1988). In FRTL-5 rat thyroid cells, TNF α induces interferon regulatory factor-1 (Mori *et al.* 1999), which plays a role in immune responses (Taniguchi *et al.* 1997). Taken together, these results suggest that TNF α may be involved in the development of autoimmune thyroiditis such as Hashimoto's thyroiditis.

While the majority of TNF α detected in inflamed thyroid tissues is produced by infiltrating inflammatory cells (Aust *et al.* 1996), studies demonstrate the production of TNF α by thyroid epithelial cells (Zheng *et al.* 1992, Mori *et al.* 1998). It may be possible that TNF α produced

by infiltrating inflammatory cells induces its own expression in thyrocytes in the area of infiltration, since autoinduction of TNF α has been demonstrated in HL-60 human promyelocytic leukemia cells and in rat tracheal epithelial cells (Spriggs *et al.* 1990, Bader & Nettesheim 1996). If so, thyrocyte-produced TNF α may stimulate infiltrating cells to aggravate inflammation in autoimmune thyroiditis. However, autoinduction of TNF α in thyroid epithelial cells has never been reported. In the present study, we examined whether TNF α induced its own expression in FRTL-5 cells.

Materials and Methods

Reagents

Recombinant rat IFN γ and recombinant mouse TNF α were obtained from Genzyme (Cambridge, MA, USA). Recombinant human TNF α was purchased from R&D Systems (Minneapolis, MN, USA). Lipopolysaccharide (LPS; *Escherichia coli* O55:B5) and amiodarone (AMD) were purchased from Sigma Chemical Co. (St Louis, MO, USA). α -³²P-dATP was purchased from New England Nuclear Corporation (Boston, MA, USA).

³⁵S-methionine (TRAN³⁵S-LABEL) was obtained from ICN Biomedicals (Irvine, CA, USA). Anti-p65 subunit of nuclear factor kappa B (NF- κ B) and preimmune rabbit serum were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Antibodies to I κ B α and phosphorylated I κ B α (Ser 32) were obtained from New England Biolabs (Beverly, MA, USA). The chemiluminescence detection system (ECL) was purchased from Amersham Biosciences (Piscataway, NJ, USA). The Superscript preamplification system containing Superscript II reverse transcriptase was obtained from Gibco/BRL (Grand Island, NY, USA). All other chemicals and reagents were obtained from commercial sources and were of reagent or molecular biology grade.

Cell culture

FRTL-5 cells were obtained from ATCC (Rockville, MD, USA), proliferated in a thyrotropin (TSH)-dependent manner and possessed all the properties previously described (Mori *et al.* 2001). Cells were grown in Coon's modified Ham's F12 medium supplemented with 5% calf serum containing bovine TSH (1 mU/ml), bovine insulin (10 μ g/ml), human transferrin (5 mg/ml), glycyl-L-histidyl-L-lysine (2 ng/ml), somatostatin (10 ng/ml) and hydrocortisone (0.36 ng/ml; 6H medium). After cells approached confluence, cells were cultured for 7 days in media devoid of TSH (5H medium). This treatment allowed cells to be quiescent as shown previously (Mori *et al.* 2001). Human thyroid epithelial cells were obtained and cultured as previously described (Trieb *et al.* 1992) with modification. Normal thyroid tissues were removed from two patients who underwent thyroidectomy for papillary carcinoma. Informed consent was obtained from each patient and the study protocol was approved by the local ethics committee. The tissues were washed with PBS and minced into small pieces. They were digested with 1 mg/ml collagenase I (Sigma Chemical Co.) and 2.4 U/ml dispase II (Roche Diagnostics GmbH, Penzberg, Germany) in PBS at 37 °C for 45 min. This procedure was repeated for 75 min with fresh enzyme mixture. The resulting cell suspension was filtered through 100 μ m mesh and washed in 6H medium. Cells were cultured in 6H medium for 15 h followed by vigorous washing to remove non-adherent and loosely adherent cells. The remaining adherent cells were detached by treatment with trypsin EDTA solution (Gibco/BRL) and plated in culture flasks and 96-well flat-bottomed plates. More than 95% cells were stained with serum containing a high titer of anti-thyroglobulin and anti-thyroid peroxidase antibodies from a patient with Hashimoto's thyroiditis (data not shown). The culture medium was changed twice a week. On the day of experiment, cells were incubated with test reagents for the indicated time and harvested for analyses. Treatment of

cells did not result in cell detachment and had no effect on cell viability, as assessed by trypan blue exclusion.

Extraction of soluble nuclear proteins

Soluble nuclear proteins were obtained as previously described (Mori *et al.* 1999). In brief, FRTL-5 cells were washed twice with ice-cold PBS, and harvested in 1 ml hypotonic buffer containing 10 mM N-(2-hydroxyethyl) piperazine-N'-(2-ethane sulfonic acid; HEPES)-KOH, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 10 μ g/ml aprotinin, 10 μ g/ml leupeptin, 1 mM NaF and 1 mM Na₃VO₄. After incubation on ice for 15 min, cells were vortexed for 10 s in hypotonic buffer containing 0.1% NP-40, followed by incubation on ice for 10 min. Nuclei were pelleted by centrifugation, washed twice with hypotonic buffer and then the nuclear pellets were incubated at 4 °C for 30 min in hypertonic buffer containing 20 mM HEPES-KOH, pH 7.9, 400 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 10 μ g/ml aprotinin, 10 μ g/ml leupeptin, 1 mM NaF, 1 mM Na₃VO₄ and 20% glycerol. Supernatants were collected after centrifugation and stored at -80 °C until use. Protein concentrations were measured by the Bradford method (Bradford 1976).

Electrophoretic mobility shift assay (EMSA)

The synthetic oligonucleotides, 5'-CAAACAGGGGGCT TTCCCTCCTC-3' and 5'-GAGGAGGGAAAGCCCC CTGTTTG-3', containing the NF κ B (κ B3) site in rat TNF α gene promoter (Nathens *et al.* 1997), were used for the detection of the protein-DNA complex by EMSA. The double-stranded probes were end-labeled using Klenow-DNA polymerase and α -³²P-dATP. Nuclear proteins (5 μ g) were incubated with 40 fmol ³²P-labeled probe at 22 °C for 30 min in buffer containing 10 mM HEPES-KOH, pH 7.9, 100 mM KCl, 0.5 mM EDTA, 0.5 mM EGTA, 0.5 mM DTT, 0.5 mM PMSF, 10% glycerol, 0.05% NP-40 and 2 μ g poly(dI-dC)-poly(dI-dC). The nucleoprotein complexes were resolved by nondenaturing electrophoresis in a 5% polyacrylamide gel at 4 °C in buffer containing 45 mM Tris-HCl, pH 8.0, 45 mM boric acid and 1 mM EDTA. Gels were dried and exposed to BioMax MS film (Eastman Kodak, Rochester, NY, USA) at -80 °C. For competition experiments, a 100-fold molar excess of the unlabeled oligonucleotides was added 15 min before incubation of nuclear extracts with radiolabeled probes. In supershift assays, nuclear proteins were incubated for 1 h at 22 °C with 1 μ l anti-p65 subunit of NF κ B or preimmune rabbit serum followed by addition of radiolabeled probes.

Western blot analysis

Cells were washed with ice-cold washing buffer containing 10 mM sodium phosphate, pH 7.4, 137 mM NaCl

Table 1 Length of amplified products, primer sequences and PCR cycles

	Length	Sequence	Cycle
Transcript			
TNF α	687	ATGAGCACGGAAAGCATGATC AGTAGACCTGCCCGGACTCCG	31
TNF-R1	322	ACCAAGTGCCACAAAGGAACC TACACACGGTGTTCTGTTCTCC	29
TNF-R2	252	ATGAGAAATCCCAGGATGCAG ACAGACGTTACGATGCAGGTG	33
GAPDH	452	ACCACAGTCCATGCCATCAC TCCACCACCCTGTTGCTGTA	22

and 1 mM Na₃VO₄ and solubilized for 30 min at 4 °C in the lysis buffer containing 50 mM Tris-HCl, pH 7.5, 137 mM NaCl, 2 mM EGTA, 1 mM PMSF, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 1 mM Na₃VO₄, 1 mM NaF and 0.1% Triton X-100. Supernatants were collected after centrifugation and aliquots containing 50 µg protein were mixed with Laemmli sample buffer and separated by SDS-PAGE (Laemmli 1970). Proteins were transferred to a nitrocellulose membrane by electroblotting. After blocking with 1% BSA in Tris-buffered saline containing 0.1% Tween 20 (TBST), membranes were incubated with rabbit polyclonal antibody to serine-phosphorylated IκBα (1:1000) overnight at 22 °C. The membranes were washed and incubated with anti-rabbit IgG conjugated with horseradish peroxidase (1:2000) for 1 h at 22 °C.

Blots were visualized using the ECL detection system. After stripping, the membranes were reprobed with rabbit polyclonal anti-IκBα (1:1000).

RT-PCR

Cells were washed twice with ice-cold PBS and total cellular RNA was isolated by the acid guanidinium-thiocyanate-phenol-chloroform extraction method of Chomczynski and Sacchi (1987), using Trizol (Gibco/BRL). Four micrograms total RNA were reverse transcribed with Superscript II reverse transcriptase according to the manufacturer's instructions. All PCRs were performed in a 50 µl reaction volume containing 1 µl aliquots from each cDNA reaction, 10 pmol of each upstream and downstream primer, 1.25 units Taq polymerase (Takara, Otsu, Japan), 0.2 mM of each dNTP, and 2 mM MgCl₂. Amplification was performed at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s followed by a 5-min extension at 72 °C. The primer sequences, product length and PCR cycles shown in Table 1 were used for the detection of TNF α , two types of TNF receptors, TNF-R1 and TNF-R2, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene expression in FRTL-5 cells. Primer pairs for rat TNF α span introns, and extra bands suggesting genomic DNA contamination were not seen. Human TNF α and human β -actin gene expression was determined using a human TNF α primer pair (BioSource International, Camarillo, CA, USA) and a human β -actin

Figure 1 Effect of TNF α or LPS on nuclear NF- κ B binding activity in FRTL-5 cells. Nuclear proteins were incubated with ³²P-labeled synthetic oligonucleotides containing the NF- κ B element (κ B3) of rat TNF α gene promoter and NF- κ B binding activity was analyzed with EMSA. Cells were stimulated with 10 ng/ml mouse TNF α or 100 µg/ml LPS for the indicated times (0, unstimulated), 10, 30, 60 min). P, ³²P-labeled probe only and C, a 100-fold molar excess of unlabeled oligonucleotides were included as competitors. The complex formed in response to TNF α or LPS was supershifted by the anti-p65 subunit of NF- κ B (p65), but not by preimmune rabbit serum (NS). The data presented are representative of three separate experiments.

Figure 2 Effect of mouse TNF α on I κ B α phosphorylation and degradation in FRTL-5 cells. Cells were incubated with 10 ng/ml mouse TNF α for the indicated times (min). Cellular proteins (50 μ g/lane) were separated by SDS-PAGE and transferred to a nitrocellulose membrane by electroblotting. Phosphorylated and total I κ B α were detected with antibodies to serine-phosphorylated and total I κ B α and by the ECL detection system. The data presented are representative of three separate experiments.

primer pair (Takara). Control reactions without reverse transcription were carried out in parallel. Cycle numbers were chosen to lie in the linear range of amplification. Rat GAPDH and human β -actin were used as an internal standard to verify that equal amounts of RNA had been subjected to RT-PCR. PCR products were separated on 1.5% agarose gel and visualized with ethidium bromide. All PCR products were cloned into pGEM-T vector (Promega, Madison, IL, USA) and sequenced by the dideoxy chain termination method.

Metabolic labeling and immunoprecipitation

TNF α protein production in TNF α -stimulated cells was assessed by immunoprecipitation of 35 S-labeled TNF α (Zhou *et al.* 2000). Briefly, cells were washed with PBS and incubated in methionine-free RPMI (ICN Bio-medicals) supplemented with L-glutamine, penicillin-streptomycin, 1% calf serum and 35 S-methionine (TRAN- 35 S-LABEL; 100 μ Ci/ml) for 24 h in the presence or absence of 50 ng/ml TNF α . Cells were washed and lysed in the lysis buffer. Samples were clarified by centrifugation and standardized to protein concentration. Cell extracts were precleared twice by incubating with protein A/G agarose (Santa Cruz Biotechnology). The samples were incubated with 5 μ g antibody to TNF α (Genzyme) or normal goat IgG overnight and protein A/G agarose was added for an additional 2 h. The beads were washed 5 times and eluted in Laemmli sample buffer. The proteins were separated by SDS-PAGE and dried gels were subjected to autoradiography.

TNF α ELISA

TNF α released into the culture medium by FRTL-5 cells treated with LPS for the indicated times was measured by a rat TNF α enzyme-linked immunosorbent assay (ELISA) kit (BioSource International), according to the manufacturer's instructions. The detection limits for rat TNF α were 0.7 pg/ml. Human TNF α was measured by a human

Figure 3 (A) Induction of TNF α gene expression by LPS in FRTL-5 cells. Cells were incubated with 100 μ g/ml LPS for the indicated times (h). Total RNA was reverse transcribed and the resulting cDNA was used for the PCR. The relative amounts of TNF α mRNA were normalized with GAPDH mRNA levels. The data presented are representative of three separate experiments. (B) Production of TNF α by LPS-stimulated FRTL-5 cells. Cells were incubated with 100 μ g/ml LPS for the indicated times (h). TNF α levels in culture medium were determined using an ELISA kit. Data are the means of triplicate culture supernatants $\pm$ s.d. and are representative of two separate experiments. n.d., not detectable. * $P < 0.05$ compared with unstimulated cells (0 h).

TNF α ELISA kit (BioSource International) with the minimum detectable level of 0.1 pg/ml. Data are the means of triplicate culture supernatants $\pm$ s.d. and are representative of two separate experiments. Statistical analysis was performed using one-way ANOVA followed by Fisher's protected least significant difference test. A level of $P < 0.05$ was considered statistically significant.

Results

EMSA was performed to verify that TNF α and LPS activate the transcription factor NF- κ B, which plays a central role in the TNF α - and LPS-stimulated intracellular signaling pathway in FRTL-5 cells. No NF- κ B binding activity was detected in unstimulated cells (Fig. 1). Mouse TNF α induced a rapid and marked increase in NF- κ B binding activity. Addition of a 100-fold molar excess of unlabeled oligonucleotides to the binding reaction completely blocked the binding activity. Furthermore, the complex formed in response to TNF α was supershifted by the anti-p65 subunit of NF- κ B, but not by preimmune rabbit serum (Fig. 1), indicating that activated NF- κ B containing the p65 subunit was induced in response to TNF α . Consistently, stimulation of cells with mouse

Figure 4 (A) Induction of TNF α gene expression in TNF α -stimulated FRTL-5 cells. Cells were incubated with 10 ng/ml mouse TNF α for the indicated times (h). The data presented are representative of three separate experiments. (B) Dose-dependent induction of TNF α gene expression in mouse TNF α -stimulated FRTL-5 cells. Cells were treated with various doses of TNF α for 2 h. The data presented are representative of three separate experiments. (C) *De novo* TNF α protein synthesis by TNF α -treated FRTL-5 cells. Cells were stimulated with 50 ng/ml mouse TNF α and metabolically labeled with ³⁵S-methionine for 24 h. Cells were lysed, and TNF α molecules were immunoprecipitated using anti-TNF α antibody or normal goat IgG. Immunoprecipitates were subjected to SDS-PAGE under reducing conditions. Gels were dried and newly synthesized TNF α proteins were visualized by autoradiography. The data presented are representative of two separate experiments.

TNF α resulted in an increase in phosphorylated I κ B α levels and a concomitant decrease in total I κ B α levels at 10 and 30 min after stimulation (Fig. 2). Similar to TNF α , LPS activated NF- κ B (Fig. 1), consistent with previous studies (Guha & Mackman 2001). Thus, we confirmed that the major signaling molecule, NF- κ B, was activated in response to TNF α and LPS in FRTL-5 cells.

TNF α gene expression in FRTL-5 cells was analyzed by semiquantitative RT-PCR. As shown in Fig. 3A, TNF α gene expression was not detected in unstimulated cells. Incubation of FRTL-5 cells with LPS, a very potent inducer of TNF α , resulted in a rapid and marked increase in TNF α mRNA levels. As a result, FRTL-5 cells released very low but detectable levels of TNF α protein in response to LPS (Fig. 3B). Thus, we confirmed that FRTL-5 cells could produce TNF α in response to stimuli such as LPS.

Similar to LPS, TNF α clearly induced its own gene expression in FRTL-5 cells (Fig. 4A). Dose-dependent induction of the TNF α gene was observed in TNF α -treated cells (Fig. 4B). We evaluated TNF α protein production by metabolic labeling and immunoprecipitation of newly synthesized TNF α protein. As shown in Fig. 4C, a modest but apparent increase in the levels of a 26 kDa protein was observed in TNF α -treated cells.

Thus *de novo* TNF α protein biosynthesis was induced in response to TNF α treatment in FRTL-5 cells. We confirmed that induction of TNF α gene was specific for LPS or TNF α since it was not observed over a 24-h incubation period in cells treated with IFN γ or AMD (data not shown), which induces interleukin (IL)-6 gene expression in the thyroid (Nakajima *et al.* 2001).

To determine whether autoinduction of TNF α gene was specific for FRTL-5 cells, human thyroid epithelial cells were stimulated with LPS or TNF α . As reported previously (Zheng *et al.* 1992), human thyrocytes produced detectable levels of TNF α protein in response to LPS stimulation (Fig. 5A). In addition, TNF α induced its own gene expression in human thyroid cells (Fig. 5B). Thus, we confirmed that autoinduction of TNF α gene expression occurs in human thyrocytes as well as in FRTL-5 cells.

Human TNF α , which does not interact with the TNF-R2 in rodents (Lewis *et al.* 1991), induced TNF α gene expression (data not shown). We tested whether upregulation of TNF-Rs was involved in autoinduction of TNF α in FRTL-5 cells. As illustrated in Fig. 6, mouse TNF α failed to increase either TNF-R1 or TNF-R2 mRNA levels.

Figure 5 (A) Production of TNF α by LPS-stimulated human thyroid epithelial cells. Cells were incubated with 100 μ g/ml LPS for the indicated times (h). TNF α levels in culture medium were determined using an ELISA kit. Data are the means of triplicate culture supernatants $\pm$ s.d. and are representative of two separate experiments. n.d., not detectable. * $P < 0.05$ compared with unstimulated cells (0 h). (B) Induction of TNF α gene expression in TNF α -stimulated human thyroid cells. Cells were incubated with 10 ng/ml human TNF α for the indicated times (h). The data presented are representative of three separate experiments.

Discussion

In the present study, we clearly demonstrate that TNF α induces its own expression in FRTL-5 cells, consistent with a previous observation that TNF α mRNA and protein levels are elevated in thyrocytes of patients with Hashimoto's thyroiditis (Zheng *et al.* 1992).

Figure 6 TNF-R1 and TNF-R2 gene expression in TNF α -stimulated FRTL-5 cells. Cells were incubated with 10 ng/ml mouse TNF α for the indicated times (h). The data presented are representative of three separate experiments.

Autoinduction of TNF α has been reported in a variety of cells (Spriggs *et al.* 1990, Bader & Nettesheim *et al.* 1996), but not in thyroid cells. Thus, the present study may be the first to show the autoinduction of TNF α in FRTL-5 cells. This phenomenon is not specific for this rat thyroid cell line since TNF α can induce its own gene expression in human thyroid epithelial cells. Although the biological significance of autoinduction of TNF α in the thyroid remains unclear, these findings suggest that thyrocytes may participate in the development of autoimmune thyroiditis through production of TNF α . Furthermore, it may be possible that TNF α produced by infiltrating inflammatory cells such as activated macrophages and lymphocytes (Sariban *et al.* 1988, Aust *et al.* 1996) stimulates thyrocytes to produce TNF α . This cytokine augments IFN γ -induced MHC class II antigen expression (Weetman & Rees 1988, Zakarija *et al.* 1988) and induces cytokines such as IL-1 and IL-6 (Nawroth *et al.* 1986, Kikumori *et al.* 1998). Taken together, TNF α produced by thyroid epithelial cells may stimulate themselves and inflammatory cells in an autocrine and/or paracrine manner to facilitate immune reaction and inflammation in autoimmune thyroiditis. In accordance with this concept, Green *et al.* (1998) have demonstrated that TNF α produced in islets plays an important role in the development of autoimmune diabetes in nonobese diabetic mice. Accordingly, inhibition of TNF α action and production would be beneficial in order to suppress inflammation in autoimmune thyroiditis. This concept is supported by a recent study by Zaccane *et al.* (2002) showing that experimental autoimmune thyroiditis induced by immunization with thyroglobulin and LPS is inhibited by soluble TNF-R1.

In the present study, TNF α gene expression was clearly induced in FRTL-5 cells in response to LPS or TNF α , as assessed by RT-PCR. In contrast, LPS- or TNF α -stimulated FRTL-5 cells produced a very small amount of TNF α protein. Taken together, our results suggest that the FRTL-5 cell line may be a suitable model to determine the regulatory mechanism involved in TNF α gene expression, but not in TNF α protein synthesis, in the thyroid. Accordingly, establishment of more sensitive methods such as the immuno-PCR (Sanna *et al.* 1995) is clearly required to analyze TNF α biosynthesis in FRTL-5 cells. Alternatively, this issue could be addressed by the establishment of thyroid-derived cells that produce much higher amounts of TNF α protein. In this regard, human thyroid epithelial cells may be suitable since LPS-stimulated cells can produce larger amounts of TNF α than can FRTL-5 cells as shown in Fig. 5A.

TNF α exerts its biological effects through binding to two distinct cell surface receptors, TNF-R1 and TNF-R2 (Lewis *et al.* 1991). Previous studies demonstrated that thyroid cells possess TNF receptors (Pang *et al.* 1989, 1996). However, TNF-R gene expression remained to be elucidated in TNF α -stimulated thyroid cells. In the present study, we demonstrate detectable levels of

TNF-R1 and TNF-R2 mRNA in unstimulated FRTL-5 cells. However, TNF α failed to increase transcript levels. These results suggest that TNF α induces its own expression without upregulating its receptor. The effects of TNF α on its receptor gene expression seem to be tissue-specific. In rat tracheal epithelial cells, TNF α downregulates TNF-R1 mRNA levels (Bader & Nettesheim 1996). In contrast, this cytokine increases TNF-R1 transcripts in rat oligodendrocytes (Dopp *et al.* 1997). Finally, human TNF α , which binds to murine TNF-R1 but not to TNF-R2 (Lewis *et al.* 1991), induced TNF α gene expression, indicating that TNF-R1 is involved in autoinduction of TNF α in FRTL-5 cells.

In summary, we demonstrate that TNF α induces its own expression in FRTL-5 cells. Although the biological significance of this phenomenon remains to be elucidated, our results suggest that thyrocyte-produced TNF α may regulate immune and inflammatory reactions in the thyroid and thus may be involved in the development of autoimmune thyroiditis such as Hashimoto's thyroiditis. Furthermore, inhibition of TNF α production in the thyroid may represent a novel approach to mitigating inflammation in autoimmune thyroiditis.

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