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# Detection and Characterization of RANK Ligand and Osteoprotegerin in the Thyroid Gland

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**Abstract** Receptor activator of NF-κB (RANK) ligand (RANKL) and osteoprotegerin (OPG) play essential roles in bone metabolism and immune responses. RANKL activates RANK, which is expressed by osteoclasts and dendritic cells (DC), whereas OPG acts as its decoy receptor. The role of RANKL and OPG in thyroid physiology is unclear. Northern analysis revealed pronounced OPG mRNA levels in normal human thyroid. By contrast, RANKL mRNA levels were most abundant in lymph node and appendix, and low in the thyroid. In the human thyroid follicular cell line XTC and in primary human thyroid follicular cells, OPG mRNA levels and protein secretion were upregulated by interleukin (IL)-1β (33-fold), tumor necrosis factor (TNF)-α (eightfold), and thyrotropin (TSH) (threefold). RANKL mRNA was stimulated in XTC by IL-1β and TNF-α, but inhibited by TSH. Conditioned medium harvested from IL-1β-treated XTC (containing high concentrations of OPG) inhibited RANKL-induced CD40 upregulation and cluster formation of DC. OPG mRNA levels were three times more abundant in surgical thyroid specimens of Graves' disease as compared to other thyroid diseases. Our data suggest that RANKL and OPG are produced in the thyroid gland by thyroid follicular cells, are regulated by cytokines and TSH, and are capable of modulating dendritic cell functions. Thus, these cytokines may represent important local immunoregulatory factors involved in the pathogenesis of autoimmune thyroid diseases. J. Cell. Biochem. 86: 642–650, 2002. 

**Key words:** autoimmune diseases; dendritic cells; osteoprotegerin; RANKL; thyroid

Members of the tumor necrosis factor (TNF) ligand and receptor (TNFR) superfamily regulate survival and apoptosis in various endocrine cells [Smith et al., 1994]. In the thyroid, TNF-α and its receptors TNFR I and II, Fas ligand (FasL) and its receptor Fas, and TNF-related apoptosis-inducing ligand (TRAIL) and its receptors TRAIL-R1 and -R2 have important roles in mediating apoptosis [Baker, 1999]. Treatment with TNF-α and IFN-γ resulted in marked induction of apoptosis in thyroid follicular cells [Bretz et al., 1999a]. FasL appears to mediate some of the effects of TNF-α [Bretz et al., 1999a], and may play a role in thyroid follicular cell apoptosis in Hashimoto's thyroiditis [Giordano et al., 1997]. More recently, thyroid follicular cells and intrathyroidal lymphocytes have been shown to express TRAIL mRNA, and TRAIL has been demonstrated to mediate apoptosis in thyroid follicular cells [Bretz et al., 1999b]. These studies have implicated TNF ligands and receptors as important cytokines in the pathogenesis of autoimmune thyroid diseases such as Hashimoto's thyroiditis and Graves' disease (GD) [Palazzo et al., 2000; Yamazaki et al., 2000].

Receptor activator of NF-κB (RANK) ligand (RANKL), a recently identified member of the TNF ligand superfamily [Anderson et al., 1997;

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Lacey et al., 1998], acts by stimulating its receptor RANK [Anderson et al., 1997]. RANKL is predominantly produced by osteoblastic lineage cells and by activated T cells, whereas RANK is mainly expressed by osteoclasts, hematopoietic precursor cells, T cells, and dendritic cells (DC) [Anderson et al., 1997; Lacey et al., 1998; Kong et al., 1999; Josien et al., 2000]. DC are the most potent antigen-presenting cells of the immune system, capable of capturing and processing antigens and subsequently initiating and modulating specific immune responses by activation of T cells [Hart, 1997; Banchereau and Steinmann, 1998]. Furthermore, DC are able to induce T cell tolerance towards self-antigens [Zal et al., 1994; Steinbrink et al., 1997], a phenomenon that is critically impaired in human autoimmune diseases such as rheumatoid arthritis or psoriasis, in which the number and activity of DC is increased [Banchereau and Steinmann, 1998]. RANKL promotes growth and survival of osteoclasts [Lacey et al., 1998; Kong et al., 1999], DC [Anderson et al., 1997; Josien et al., 2000], and mammary epithelial cells [Fata et al., 2000] by preventing their apoptosis. Physiologically, the effects of RANKL are counteracted by the decoy receptor osteoprotegerin (OPG), which binds RANKL, thus preventing RANKL–RANK interactions [Simonet et al., 1997]. Of note, OPG also acts as a decoy receptor for TRAIL and prevents the apoptosis-inducing effects of TRAIL [Emery et al., 1998]. Emerging evidence suggests that RANKL, RANK, and OPG act as important regulators of the immune system [Kong et al., 2000]. An imbalance of this cytokine system with an altered RANKL-to-OPG ratio has been implicated in the pathogenesis of animal models of rheumatoid arthritis [Kong et al., 1999], osteolytic tumor metastases [Honore et al., 2000], humoral hypercalcemia of malignancy [Capparelli et al., 2000], and various human metabolic bone diseases [Hofbauer and Heufelder, 2000b]. By contrast, exogenous administration of OPG prevented these disorders [Kong et al., 1999; Capparelli et al., 2000; Honore et al., 2000; Hofbauer and Heufelder, 2000b].

To date, the roles of RANKL and OPG in thyroid physiology have not been assessed. DC are present in the thyroid gland and play an important role in the pathogenesis of autoimmune thyroid diseases and thyroid carcinoma [Yamakawa et al., 1995; Kitahama et al., 1996]. Since, TNF ligand superfamily members TNF-α, FasL, and TRAIL are potent mediators in the pathogenesis of autoimmune thyroid diseases, we evaluated the role of the novel TNF superfamily ligand RANKL and its decoy receptor OPG in thyroid cell biology and in autoimmune thyroid diseases. Our data indicate that the thyroid gland is a source of RANKL and OPG, which are modulated by cytokines and thyrotropin (TSH) and overexpressed in GD, and that thyrocyte-derived OPG may modulate DC functions.

## MATERIALS AND METHODS

### Materials

Culture flasks and dishes were obtained from Corning (Corning, NY), cell culture medium was purchased from Sigma (St. Louis, MO). The random primer labeling kit (Decaprime II) was from Ambion (Austin, TX) and [α-$^{32}$P]-dCTP from DuPont-NEN (Boston, MA). The human β-actin cDNA insert, ExpressHyb solution and commercial poly-A and Northern dot blots were purchased from Clontech (Palo Alto, CA). All cytokines were from R&D Systems (Minneapolis, MN).

Thyroid specimens were obtained from patients undergoing thyroid surgery after written informed consent and stored at $-80^{\circ}$C until used. All studies involving human subjects were approved by the Institutional Review Board of Philipps University, Marburg.

### Cell Cultures

We employed the differentiated thyroid follicular cell line XTC as a cell model of thyroid follicular cells [Zielke et al., 1998]. XTC cells were grown in DME-h21/Ham's F12 medium (1:1) (v/v) supplemented with HEPES (25 mM), sodium pyruvate (0.5 mM), glutamine (2.5 mM), 10% (v/v) fetal bovine serum (FCS), penicillin, and streptomycin at 37°C. Forty-eight hours prior to RNA isolation, XTC cells were cultured in serum-free medium supplemented with bovine insulin (10 μg/ml), human transferrin (5 μg/ml), somatostatin (10 ng/ml), and glycyl-L-histidyl-L-lysine (2 ng/ml) as described [Zielke et al., 1999]. XTC cells display the phenotype of differentiated follicular thyroid cells following treatment with TSH, including increased proliferation rates and enhanced thyroglobulin secretion [Zielke et al., 1999]. Primary thyroid follicular cells were derived from surgical speci-

mens of patients undergoing thyroidectomy. Thyroid tissues were mechanically dissected, treated by collagenase digestion, and cultured in DMEM/Ham's F12 medium supplemented with 10% (v/v) FCS, penicillin, and streptomycin at 37°C as previously described [Grubeck-Loebenstein et al., 1989].

### Northern Blot Analysis

Total RNA was isolated using the RNeasy kit and the QiaShredder from Qiagen (Hilden, Germany). Poly-A RNA was isolated using the PolyATract mRNA kit from Promega (Madison, WI). Ten micrograms of total RNA or 2 μg of poly-A RNA were separated on a 1.5% (w/v) agarose/formaldehyde gel and subsequently transferred to a nylon membrane (Hybond N+, Amersham, Arlington Heights, IL) by capillary blotting. The human cDNA inserts, a β-actin cDNA that hybridized to a 2.0 kb mRNA, a full-length OPG cDNA that hybridized to three mRNA species of 2.9, 4.4, and 6.6 kb, respectively, and a RANKL cDNA that hybridized to a mRNA species of 2.4 kb were radiolabeled using a random primer DNA labeling kit. Hybridization procedures were carried out as previously described [Hofbauer et al., 1999a]. All experiments were repeated at least three times with similar results, and representative blots are shown. Control hybridizations with human β-actin cDNA verified that equal amounts of RNA were loaded.

### OPG Protein Measurement

Conditioned medium was harvested from cultured cells and centrifuged to remove cell debris. OPG protein concentrations were determined in triplicate measurements using a sandwich ELISA as described previously following 1:20 to 1:100 dilution [Hofbauer et al., 1999a].

### Isolation of DC and Culture Conditions

DC were generated from blood monocytes following standard procedures [Romani et al., 1996]. In brief, peripheral blood mononuclear cells (PBMC) from healthy volunteers were obtained by Ficoll density gradient centrifugation (Biochrom, Berlin, Germany) and resuspended in RPMI 1640 medium (Sigma), supplemented with 10% (v/v) heat-inactivated FCS, penicillin, and streptomycin (complete medium). Subsequently, the cells were incubated in Petri dishes at a density of $5 \times 10^6$ cells/ml. The nonadherent cell fraction was removed 1 h later, and adherent cells were cultured in complete medium supplemented with GM-CSF (1,000 U/ml) and IL-4 (800 U/ml) for 7 days. On day 3 and 5, fresh cytokines were added, and on day 7 half of the volume of medium was replaced by fresh complete medium supplemented with IL-1β (1,000 U/ml), IL-6 (1,000 U/ml), TNF-α (1,000 U/ml), and prostaglandin $E_2$ ($10^{-8}$ M). Mature DC were collected on day 10 and further cultured in 24-well-plates at $2 \times 10^5$ cells/ml in complete medium supplemented with soluble RANKL (1 μg/ml) or negative control (PBS/BSA vehicle) in a total volume of 500 μl. RANKL—at this concentration—has previously been identified as a survival factor for DC in vitro [Wong et al., 1997]. To test the biological effects of thyrocyte-derived OPG, conditioned medium from XTC treated with either IL-1β at a concentration of 5 nM (which contained high levels of OPG protein) or vehicle was used after 10-fold concentration through cold centrifugation. DC were treated with XTC-derived conditioned medium supplemented with RANKL at 1 μg/ml.

### Flow Cytometry

DC were analyzed for cell surface expression of CD40 by fluorescence-activated cell sorting using a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany) [Wong et al., 1997]. The monoclonal antibody was purchased from PharMingen (Hamburg, Germany). CD40 staining of DC was followed by incubation with a FITC-conjugated goat anti-mouse antibody (Dianova, Hamburg, Germany). A minimum of 8,000 events were analyzed per sample. DC morphology and ability to form clusters were assessed microscopically, and photographs were taken from representative fields. Cell viability was also assessed by FACS analysis.

### Statistical Analysis

Unless otherwise stated, all values are expressed as the mean ± SEM. Student's paired *t*-test was used to evaluate differences between the sample of interest and its respective control. All tests were two-tailed. For analysis of dose responses and time courses, multiple measurement ANOVA was used. A $P$ value of $< 0.05$ was considered statistically significant.

## RESULTS

To compare the level of expression of OPG and RANKL mRNA among several endocrine and

non-endocrine tissues, a commercial multi-tissue dot blot was hybridized with OPG and RANKL probes [Hofbauer et al., 1998, 1999b]. Northern blot analysis demonstrated the presence of high OPG mRNA levels in the thyroid gland, aorta, and kidney. By contrast, RANKL mRNA was most abundant in lymph node, appendix, and fetal liver, and only low RANKL mRNA levels were present in adrenal gland, mammary gland, and thyroid gland (data not shown). To further analyze OPG mRNA expression and to confirm the dot blot findings, hybridization using a commercial poly-A Northern blot with various endocrine tissues was performed. Marked OPG mRNA expression was detected in the thyroid gland (a major 2.9-kb species and two minor bands at 4.4 and 6.6 kb, respectively), whereas low OPG mRNA steady state levels were detected in adrenal medulla and cortex, and pancreas (Fig. 1). These studies demonstrated abundant OPG gene expression in the thyroid gland, which exceeded the amount in other endocrine tissues and prompted us to further study the potential role of the RANKL/OPG system in thyroid cell physiology.

To assess the origin of OPG gene expression detected in poly-A RNA isolated from whole thyroid glands and to evaluate its modulation by thyrotropic agents, we employed a differentiated follicular thyroid cell line (XTC). The pro-inflammatory cytokines IL-1β and TNF-α induced OPG mRNA steady state levels in XTC cells from low baseline levels dose-dependently by more than 20-fold (IL-1β at a concentration of 5 nM) and eightfold (TNF-α at a concentration of 9 nM), respectively (Fig. 2). A similar dose-dependent induction of OPG protein secretion by IL-1β was observed in the conditioned medium harvested from XTC cells (33-fold increase at 5 nM, $P < 0.0001$ by ANOVA, Fig. 3A). Moreover, TNF-α stimulated OPG protein secretion of XTC in a time-dependent fashion (fourfold increase after 24 h, $P < 0.001$ by ANOVA, Fig. 3B). TSH also induced OPG mRNA steady state levels and OPG protein secretion in a dose-dependent fashion by up to threefold at a concentration of 10 mU/ml ($P < 0.005$ by ANOVA, Fig. 4). To confirm that OPG regulation by thyrotropic agents was not limited to the thyroid follicular cell line XTC, we employed primary human thyroid follicular cells derived from surgical specimens obtained during subtotal thyroidectomy. At their maximal effective concentrations, IL-1β (5 nM), TNF-α (9 nM), and TSH (10 mU/ml) stimulated baseline OPG protein secretion (2.19 ng/ml) by 2.2-, 2.7-, and 1.7-fold, respectively ($P < 0.01$ by Student's $t$-test). Of note, IL-1β (5 nM) and TNF-α (9 nM) also induced RANKL mRNA steady state levels in XTC cells by three- and fourfold, respectively, whereas TSH (10 mU/ml) decreased RANKL mRNA steady state levels by 60% as assessed by densitometry (Fig. 5).

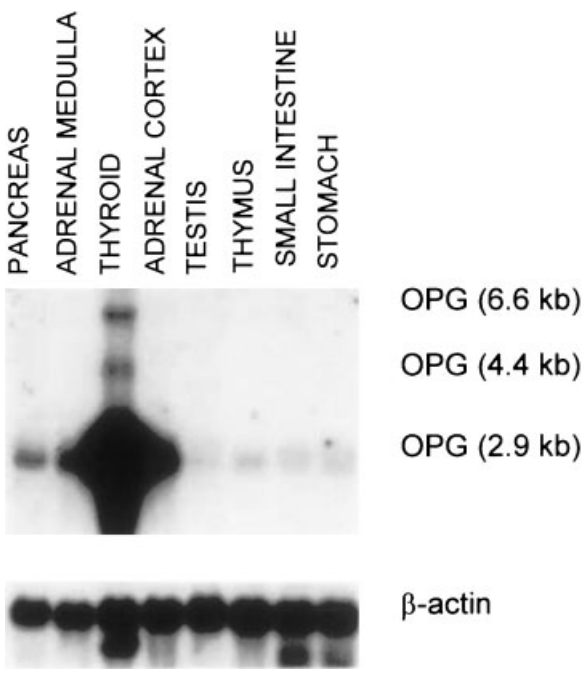


**Fig. 1.** OPG mRNA steady state levels in various endocrine tissues. A commercial multi-tissue poly-A Northern blot was hybridized with radiolabeled OPG and β-actin cDNA. OPG mRNA (2.9, 4.4, 6.6 kb) and β-actin mRNA (2.0 kb).

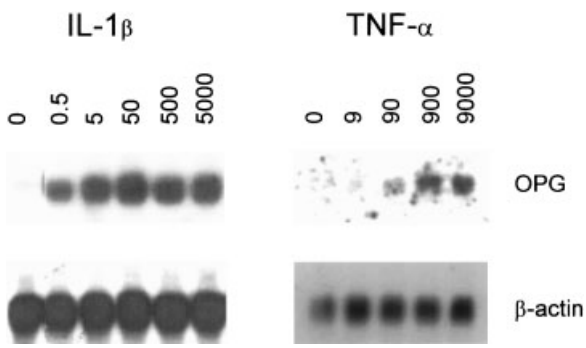


**Fig. 2.** Stimulation of OPG mRNA steady state levels in XTC cells by interleukin-1β (IL-1β) and TNF-α. XTC were treated with various concentrations of IL-1β or TNF-α for 24 h, and 10 μg total RNA were analyzed by Northern hybridization. OPG mRNA (2.9 kb) and β-actin mRNA (2.0 kb). The numbers indicate the cytokine dose in pM.

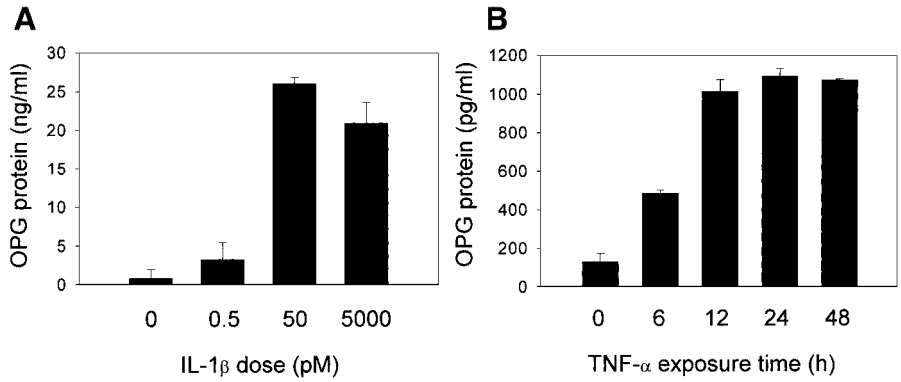


**Fig. 3.** Stimulation of OPG protein secretion in XTC cells by IL-1β and TNF-α. (**A**) XTC were treated with various concentrations of IL-1β for 24 h as described in Figure 2. (**B**) XTC were treated with TNF-α (9 nM) for various time points (indicated in h). OPG protein secretion was determined in the conditioned medium by ELISA. Values represent the mean ± SEM from triplicate measurements expressed as percentage of controls. (A) $P < 0.0001$ by ANOVA, (B) $P < 0.001$ by ANOVA.

Since OPG protein secretion was induced by IL-1β in XTC and primary human thyroid follicular cells, and because RANKL and OPG influence DC biology [Anderson et al., 1997; Wong et al., 1997; Josien et al., 2000], we next hypothesized that increased OPG secretion by XTC might modulate DC function, thus providing a potential cellular target for intrathyroidal OPG production. To study the effects of XTC-derived supernatant on mature DC after stimulation with either vehicle or IL-1β (the latter of which contained 33-fold higher OPG protein levels as demonstrated in Fig. 3A) in the presence or absence of RANKL (1 μg/ml), cells were incubated for 2 days at 37°C with the respective medium after it had been concentrated by 10-fold. Mature DC generated from blood monocytes were characterized by specific surface expression of CD40 using FACS analysis. In the absence of RANKL, DC displayed robust baseline levels of CD40 expression (Fig. 6A). Conditioned medium from vehicle-treated XTC (supplemented with RANKL) further enhanced CD40 expression by DC (Fig. 6B), whereas conditioned medium from IL-1β-treated XTC (which contained high levels of OPG) partially reversed CD40 expression by DC (Fig. 6C). Moreover, DC formed densely packed clusters in the presence of RANKL when treated with conditioned medium from vehicle-treated XTC (Fig. 6D), while treatment with conditioned medium from IL-1β-treated XTC

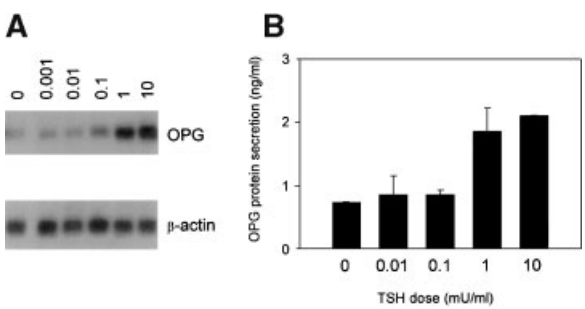


**Fig. 4.** Dose-dependent stimulation of OPG mRNA steady state levels and protein secretion in the thyroid follicular cell line XTC by TSH. (**A**) XTC were treated with various concentrations (the numbers indicate the dose in mU/ml) of TSH for 24 h at 37°, and 10 μg total RNA were analyzed by Northern hybridization. OPG mRNA (2.9 kb) and β-actin mRNA (2.0 kb). (**B**) Conditioned medium was harvested from the XTC treated as described in (A), and OPG concentrations were determined by ELISA. Values represent the mean ± SEM from triplicate measurements expressed as percentage of controls. $P < 0.005$ by ANOVA.

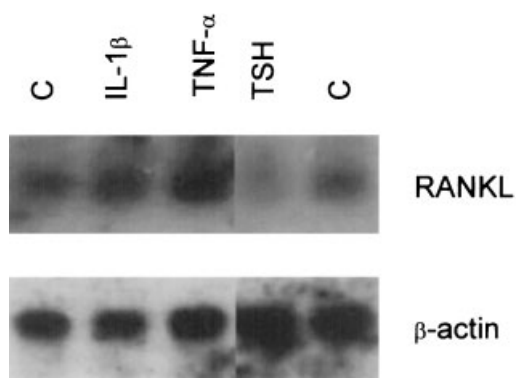


**Fig. 5.** Modulation of RANKL mRNA steady state levels in the thyroid follicular cell line XTC. XTC cells were treated for 24 h with either vehicle control (PBS + BSA, **C**), IL-1β (5 nM), TNF-α (9 nM), or TSH (10 mU/ml), and 2 μg of poly-A RNA were analyzed by Northern hybridization. RANKL mRNA (2.4 kb) and β-actin mRNA (2.0 kb).

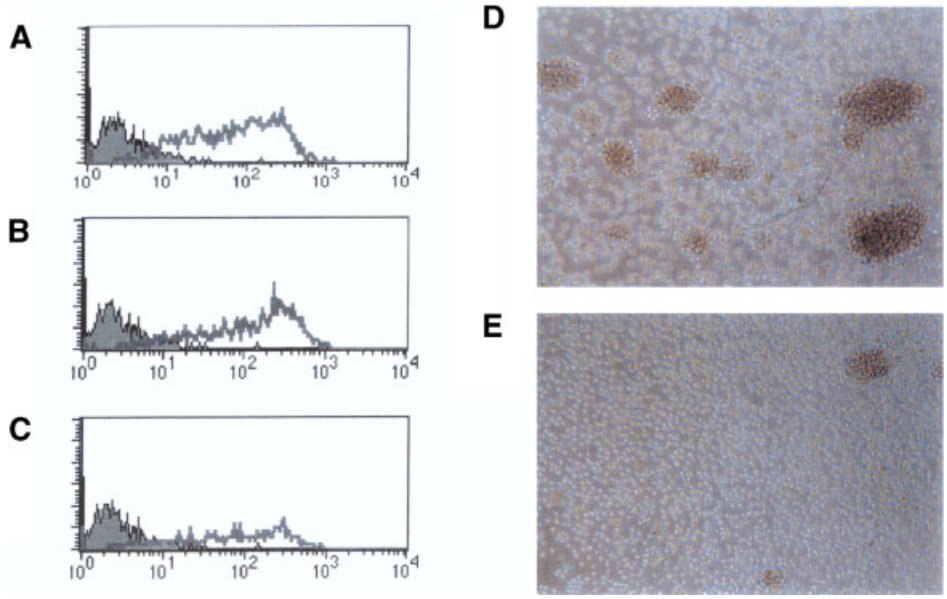


**Fig. 6.** Effects of XTC-derived OPG on DC generated from blood monocytes. (**A–C**) Characteristic FACS profile of CD40 expression (white histograms) on mature DC after 2 days of culture. A: Control (no conditioned medium, in the absence of RANKL and OPG): (B-C) Effects of conditioned medium from vehicle-treated XTC (with low OPG concentrations; (B) and IL-1β-treated XTC (with high OPG concentrations; (C), both in the presence of RANKL (1 μg/ml). Isotype-matched control antibodies represent background staining (dark histograms). y axis: Relative cell number; x axis: Log fluorescence intensity. (**D–E**) Cluster formation of DC following RANKL treatment in the presence of conditioned medium from vehicle-treated XTC (D) or IL-1β-treated XTC (E). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

containing high levels of OPG caused the formation of sparsely scattered aggregates (Fig. 6E). Thus, OPG secreted by thyroid follicular cells was able to block the RANKL-induced upregulation of CD40 and cluster formation of DC. There were no statistically significant differences in cell viability between DC treated as described in Figure 6D,E (data not shown).

We then hypothesized that increased intrathyroidal production of pro-inflammatory cytokines might modulate OPG production in vivo. To address this issue, we analyzed OPG mRNA expression in surgical thyroid tissue specimens from patients with various benign thyroid diseases after confirmation of histopathological diagnosis. Of the 35 specimens evaluated, thyroid glands derived from patients with GD revealed three- to fivefold higher OPG mRNA levels as compared to thyroid glands from patients with Hashimoto's thyroiditis, autonomous thyroid adenoma, or non-toxic multinodular goiter (Fig. 7).

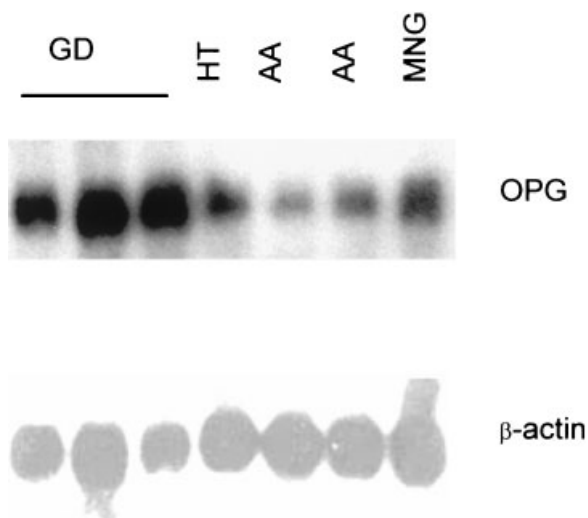


**Fig. 7.** OPG mRNA steady state levels in thyroid glands obtained from patients with benign thyroid disorders. Surgical specimens were homogenized and 10 μg of total RNA were analyzed by Northern hybridization. OPG mRNA (2.9 kb) and β-actin mRNA (2.0 kb). GD, Graves' disease; HT, Hashimoto's thyroiditis; AA, autonomous adenoma; MNG, multinodular goiter.

## DISCUSSION

Members of the TNF ligand superfamily such as TNF-α, FasL, and TRAIL are critical regula-

tors of cell differentiation, proliferation, survival, and apoptosis in various endocrine systems [Smith et al., 1994; Baker, 1999]. These cytokines are produced by intrathyroidal epithelial and immune cells, and have been implicated in impaired thyroid function in autoimmune thyroid diseases [Giordano et al., 1997; Bretz et al., 1999a,b; Yamazaki et al., 2000]. To date, the roles of the TNF ligand RANKL and its decoy receptor OPG in thyroid physiology and pathology have remained undefined. In the present study, we characterized the TNF ligand and receptor superfamily members RANKL and OPG in the thyroid gland and identified this organ as a tissue rich in OPG gene expression, with OPG mRNA steady state levels exceeding those of other endocrine organs. Moreover, we report that RANKL mRNA as well as OPG mRNA and protein are produced by thyroid follicular cells and modulated by pro-inflammatory and immunoregulatory cytokines (TNF-α, IL-1β) and TSH. Stimulation of OPG by these agents was detected regardless of the cell culture technique, stage of confluence, or cell passage, and was consistently reproduced both at the mRNA and protein level. The induction of OPG was substantial in magnitude (up to 33-fold), occurred in a dose- and time-dependent fashion, and was present both in the clonal XTC cell line and primary non-clonal human thyroid follicular cells. Moreover, stimulation of OPG concentrations by these agents (from 0.78 to 26 ng/ml by IL-1β) corresponds to the steep part of the OPG dose response curve [Simonet et al., 1997; Lacey et al., 1998], indicating that thyrocyte-derived OPG protein concentrations are within the biologically relevant dose range. Thus, stimulation of OPG production by thyroid follicular cells following treatment with IL-1β, TNF-α, and TSH meets the criteria for a physiological response.

Treatment of XTC cells with the pro-inflammatory cytokines TNF-α and IL-1β stimulated both RANKL mRNA and OPG mRNA and protein synthesis. Induction of RANKL and its decoy receptor OPG by these cytokines was previously shown in human bone marrow stromal cells and osteoblastic cells [Hofbauer et al., 1998, 1999b], and is thought to represent a safeguard against the capacity of RANKL to induce osteoclast differentiation and activation, and to promote bone loss [Hofbauer et al., 2000a]. The importance of ubiquitous OPG production despite restricted RANKL production may relate to the fact that RANKL is produced by circulating activated T cells and exists both in a membrane-bound and secreted form, suggesting the importance of OPG as a local defense and control mechanism [Hofbauer and Heufelder, 2000b; Kong et al., 2000]. Of interest, TSH, the major thyrotropic peptide hormone controlling function, proliferation, and differentiation of thyroid follicular cells, also enhanced OPG mRNA and protein production, but in contrast to the pro-inflammatory cytokines, suppressed RANKL mRNA levels. OPG production by osteoblastic lineage cells was shown to increase with their stage of differentiation [Gori et al., 2000], and to be induced by factors that promote osteoblastic differentiation such as vitamin D and bone morphogenetic protein-2 [Hofbauer et al., 1998].

In addition to osteoclastic lineage cells, RANK, the specific receptor for RANKL is expressed by DC [Anderson et al., 1997; Josien et al., 2000; Kong et al., 2000], and DC represent an established target for RANKL and OPG [Wong et al., 1997; Josien et al., 1999]. RANKL is known to enhance survival and to promote the immunostimulatory capacity of DC, whereas OPG represents the specific antagonist that counteracts this effect [Wong et al., 1997; Josien et al., 1999; Kong et al., 2000]. DC are physiologically located within the thyroid gland, and thought to contribute to the pathogenesis of autoimmune thyroid diseases by presenting intrathyroidal autoantigens and promoting chronic processes of autoimmunity, inflammation, and self-destruction [Weetman, 2000]. Our study provides the first experimental evidence for a potential paracrine mechanism of OPG within the thyroid gland. RANKL has been found to specifically upregulate surface expression of CD40 on mature DC, but not that of other co-stimulatory molecules (CD80, CD86) or adhesion molecules (ICAM-1, CD11b, CD11c) [Wong et al., 1997]. To verify whether OPG, which is abundantly expressed following IL-1β treatment of thyroid follicular cells, exerts biological effects in vitro, mature DC were assessed for CD40 expression and cluster formation in the presence of OPG and RANKL. Interestingly, OPG-containing supernatant was able to interfere with RANKL-induced upregulation of CD40 and cluster formation of mature DC, indicating that thyrocyte-derived OPG is biologically active and capable of altering certain DC functions. This paracrine

interaction may represent a local mechanism to achieve DC homeostasis within the thyroid gland. The percentage of viable cells was statistically not significantly different between DC incubated with XTC-derived conditioned medium treated with vehicle or IL-1β (data not shown), suggesting that IL-1β concurrently modulated the production of other cytokines involved in DC survival and apoptosis. Clearly, further studies are required to evaluate this in more detail.

In GD, increased DC activity is thought to contribute to the production of TSH receptor auto-antibodies and pro-inflammatory cytokines such as TNF-α and IL-1β [Weetman, 2000]. Our observation of increased intrathyroidal OPG gene expression in GD and that of a marked stimulation of OPG gene expression and protein secretion in thyroid follicular cells by those pro-inflammatory cytokines (TNF-α, IL-1β) that are overproduced locally in GD [Baker and Fosso, 1993; Weetman et al., 1997; Rasmussen et al., 2000] suggest that increased intrathyroidal OPG mRNA levels in GD may be due to the abundant intrathyroidal production of IL-1β and TNF-α. Induction of OPG by TNF-α and IL-1β may act to locally control function and activity of DC [Baker and Fosso, 1993; Weetman et al., 1997; Paolierit et al., 1999; Rasmussen et al., 2000]. This phenomenon could be due to sequestration of OPG by its heparin-binding affinity. In summary, our data indicate that the thyroid gland represents both a site of production and a potential target for the RANKL/OPG cytokine system. RANKL and OPG are produced by thyroid follicular cells, are upregulated by pro-inflammatory cytokines, and act to modulate DC functions, indicating an important local immunoregulatory network that may contribute to the pathogenesis of autoimmune thyroid diseases.

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