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**IL-14 and IL-16 are expressed in the thyroid of patients with either Graves'
disease or Hashimoto's thyroiditis**

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Abstract

Objectives Cytokines have an important role in orchestrating the pathophysiology in autoimmune thyroid disease. The aim of the current study was to analyse the expression of interleukin (IL)-14 and IL-16 in the thyroid tissue of patients with Graves' disease (GD), Hashimoto's thyroiditis (HT) or multi-nodular goitre (MNG) and in that of normal individuals, in patients' intra-thyroidal $CD4^+$ and $CD8^+$ T cells, and in patient and normal cultured thyroid follicular cells.

Methods The expression of IL-14 and IL-16 mRNA and protein was investigated using reverse transcription-polymerase chain reaction (RT-PCR) amplification, and western blotting and ELISAs, respectively.

Results IL-14 mRNA expression was detected in thyroid tissue from 8/9 GD, 3/4 HT and 3/13 MNG patients and 1/6 normal individuals, and IL-16 mRNA expression in thyroid tissue from 9/9 GD, 4/4 HT and 9/13 MNG patients and 4/6 normal individuals. IL-14 mRNA expression was detected in intra-thyroidal $CD4^+$ and $CD8^+$ T cells from 2/2 GD and 2/2 HT patients, while IL-16 mRNA was detected in samples from 1/2 HT but not in those from either GD patients. IL-14 and IL-16 mRNA expression was found in thyroid follicular cells derived from 2/2 patients with GD and 1/1 normal individual.

IL-14 protein was detected in thyroid tissue from 6/6 GD, 1/1 HT and 0/6 MNG patients and 0/6 normal individuals, and IL-16 protein in thyroid tissue from 6/6 GD, 1/1 HT and 1/6 MNG patients and 0/6 normal individuals. Expression of IL-14 protein was stimulated in thyroid follicular cells derived from two GD patients and one normal individual by peripheral blood mononuclear cell (PBMC)-conditioned medium. Treatment of thyrocytes from two GD patients and one normal individual with PBMC-conditioned

medium and tumour necrosis factor (TNF)-α stimulated IL-16 protein expression. In normal

thyrocytes, IL-16 protein synthesis was induced also by IL-1β, IL-17A, IL-4 and transforming

growth factor (TGF)-β.

Conclusions The data provide evidence that the intra-thyroidal production of IL-14 and IL-16

is associated with the pathogenesis of autoimmune thyroid disease. Thyroid follicular cells

display the ability to express IL-14 and IL-16 mRNA and can be stimulated to express IL-16

protein, by a panel of cytokines, and IL-14 protein, by as yet unidentified factors.

Introduction

Graves' disease (GD) and Hashimoto's thyroiditis (HT) are the two most common clinical

expressions of thyroid dysfunction due to autoimmunity.[1] HT is characterised by infiltration

of thyroid-specific T lymphocytes and other immune cells, thyroid enlargement and fibrosis,

and progressive destruction of thyrocytes, eventually resulting in hypothyroidism.[2] Patients

with GD may show lymphocytic infiltration and some damage to the thyroid, but the disease

is characterized by hyperthyroidism due to excessive production of thyroid hormone

induced by specific autoantibodies against the thyrotropin receptor.[3] Although several

factors, including genetic, hormonal, and environmental, have been implicated in the

initiation and/or development of thyroid autoimmunity,[4] the changes associated with the

pathophysiology of autoimmune thyroid disease are orchestrated by an array of cytokines.[5]

Particularly, pro-inflammatory Th1- and Th17-specific cytokines, which can be produced by

thyroid follicular cells as well as thyroid-infiltrating T lymphocytes, have a role in promoting

thyroid autoimmunity.[6-9]

Interleukin (IL)-14 is produced mainly by T lymphocytes and acts to stimulate B cell

proliferation, expand selected B cell subgroups, and inhibit antibody secretion.[10] It has been

implicated in the pathogenesis of autoimmune diseases, systemic lupus erythematosus and

Sjögren's syndrome.[11,12] Given the importance of the humoral immune response in

autoimmune thyroid disease, particularly GD, IL-14 may be directly involved in initiation

and/or progression of the autoimmune process. In contrast, IL-16 is involved in cellular

immune responses[13,14] and is produced by several cell types including epithelial cells,

fibroblasts, monocytes, lymphocytes and eosinophils.[15-18] This cytokine plays a role in a

number of auto-inflammatory conditions, including rheumatoid arthritis, Crohn's disease

and systemic lupus erythematosus.[19-21] Moreover, previous evidence suggests a role for IL-16 in thyroid autoimmunity.[22,23]

Given the potential for involvement of IL-14 and IL-16 in autoimmune thyroid disease pathogenesis, the aim of the current study was to analyse the expression of IL-14 and IL-16 in the thyroid tissue of patients with either GD, HT or multi-nodular goitre (MNG) and in that of normal individuals, in intra-thyroidal $CD4^+$ and $CD8^+$ T cells derived from GD and HT patients, and in primary thyrocytes taken from diseased and normal thyroids, as well as in the human thyroid cell line HT-ori3. Overall, this study will aim to establish the role of IL-14 and IL-16 in autoimmune thyroid disease which is an important step to fully understand disease pathogenesis and uncover potential future therapeutic targets.

Materials and methods

Patients and thyroid tissue samples

Local ethical committee approval was obtained for the study and informed written consent was obtained from all participants prior to inclusion in the study. Disease diagnosis was confirmed in all GD (n = 9), HT (n = 4) and non-toxic, non-autoimmune MNG (n = 13) patients by biochemical testing and histological examination. All HT patients had high antibody titres against thyroid peroxidase, whereas seven out of nine GD patients had raised anti-thyroid peroxidase antibody titres, with data unavailable for the remaining two. All patients with HT and GD had been treated with thyroxine and carbimazole, respectively, prior to surgery. Surgery in the HT patients was performed either for diagnostic purposes, because lymphoma was suspected (but not subsequently confirmed) or to remove a painful or a cosmetically disfiguring goitre. Patient thyroidectomy specimens were either snap-frozen in liquid nitrogen or processed to obtain either thyroid-infiltrating immune cells or thyroid follicular cells. Snap-frozen thyroid tissue samples were also obtained from six normal individuals (ProteoGenex, Culver City, CA, USA).

Isolation of intra-thyroidal CD4$^+$ and CD$^+$ T cells

Intra-thyroidal lymphocytes were prepared from GD and HT patient thyroidectomy specimens by collagenase/Dispase digestion as described previously.[24] CD4$^+$ and CD8$^+$ T cells were isolated using magnetic Dynabeads® M-450 beads (Dynal Biotech, Oslo, Norway), according to the manufacturer's protocol.

Thyroid follicular cells

The human thyroid cell line HT-ori3 was established by transfection of normal human thyrocytes with a plasmid-borne origin-defective SV40 genome, as previously described.[25] Thyroid follicular cells were prepared from GD patient thyroidectomy specimens using methods described elsewhere.[26] Normal primary thyrocytes were obtained from Applied Biological Materials Inc. (Richmond, BC, Canada). Thyrocytes were cultured as detailed before.[27]

Reverse transcription-polymerase chain reaction (RT-PCR) amplification

RNA was extracted from thyroid tissue, intra-thyroidal lymphocytes and cultured thyrocytes using TRIZOL® LS reagent (Life Technologies Ltd., Paisley, UK) according to the manufacturer's protocol. Moloney murine leukaemia virus reverse transcriptase (MMLV-RT; Promega, Southampton, UK) was used to synthesise cDNA, as previously described.[27] Oligonucleotide primers (Life Technologies Ltd.) for PCR amplification of cDNA samples were designed according to previously published DNA sequences of IL-14[10] and IL-16.[13] The primer sequences were 5'-CAGCTCATACTGCTCATTCA-3' (forward) and 5'-TCCTACAGAAAAAGCAGAGC-3' (reverse) for IL-14, and 5'-ATTCACTCATACATCTGGCC-3' (forward) and 5'-TGCTTCACCTGTACTCCTCT-3' (reverse) for IL-16. The primer sequences for β−actin have been described previously.[27] PCR amplification reactions were performed as detailed earlier,[6] with 27-29, 30-32 and 22-24 cycles used for IL-14, IL-16 and β-actin amplification, respectively. Control PCR amplifications without cDNA were carried out in parallel and were consistently negative. PCR amplification products were separated in 1% (w/v) agarose gels and their identification confirmed by Southern blotting and hybridisation using internal oligonucleotide probes (Life Technologies Ltd.), as previously described.[27]

Probes used were 5'-TCAGCCAGCTCCATGTTC-3' for IL-14,[10] and 5'-TGAATGCTTATAGGTGGC-3' for IL-16.[13] The probe for β-actin has been detailed elsewhere.[24]

Preparation of protein extracts from thyroid tissue and cultured thyrocytes

Proteins were extracted from thyroid tissue and cultured thyroid follicular cells using TRIZOL® LS reagent (Life Technologies Ltd.) according to the manufacturer's protocol. Protein concentrations were determined using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK).

Western blotting

Equivalent amounts of total protein in thyroid tissue or thyrocyte extracts were separated in SDS-polyacrylamide gels and transferred to Whatman™ Westran® Clear Signal polyvinylidene difluoride membrane (GE Healthcare Life Sciences, Little Chalfont, UK) using standard techniques. After treating membranes with Blocking Reagent (Roche Diagnostics, Mannheim, Germany), primary anti-IL-14 (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) or anti-IL-16 (Santa Cruz Biotechnology, Inc.) antibody was applied to them at the required dilution in PBS/0.5 % (v/v) Blocking Reagent (Roche Diagnostics) before incubation with shaking at room temperature for 1.5 h. Secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Inc.) at the required dilution in PBS/0.5 % (v/v) Blocking Reagent was applied to washed membranes prior to incubating them for 1 h at room temperature with shaking. Following washing, binding of the secondary antibody to the membranes was detected using a BM Chemiluminescence Blotting Substrate (POD) Kit (Roche Diagnostics) and exposure to X-ray film, as explained in the manufacturer's manual.

Cytokine treatment of cultured thyroid follicular cells

For analysing the effects of cytokines and TSH on IL-14 and IL-16 protein expression, thyroid follicular cell cultures were grown to confluence in 25-cm^2 flasks and were then left untreated or treated for 6, 12 and 24 h with either IL-1α (100 U/ml; Hoffman-La Roche Inc., Nutley, NJ, USA), bovine TSH (100 μU/ml; National Institute for Biological Standards and Control, Potters Bar, UK), interferon (IFN)-γ (100 U/μl; Roche Diagnostics GmbH, Mannheim, Germany), tumour necrosis factor (TNF)-α (100 U/ml; CN Biosciences Ltd., Nottingham, UK), IL-1β (10 ng/ml), IL-17A (20 ng/ml), IL-4 (10 ng/ml) or transforming growth factor (TGF)-β (10 ng/ml) (all from R&D Systems, Inc., Minneapolis, MN, USA), as detailed previously.[27] To provide a positive control for the induction of IL-14 and IL-16 protein expression, peripheral blood mononuclear cells (PBMC) from a healthy individual were treated with 1 μg/ml of phytohaemagglutinin M (Life Technologies Ltd.) for 24 h and the conditioned culture medium centrifuged at 500 *g* for 10 min, sterilised by filtration, and then used to treat cultured thyrocytes for 6, 12 and 24 h. Samples of thyroid follicular cell culture supernatants were collected prior to treatment at 0 h and at the stated time points, centrifuged at 500 *g* for 10 min, and then analysed for IL-14 and IL-16 protein levels in IL-14- or IL-16-specific ELISAs, respectively. After 24 h, untreated and treated thyrocytes were collected for the preparation of protein extracts, as detailed above.

Cytokine ELISAs

IL-14- and IL-16-specific ELISAs were used according to the manufacturers' protocols. The IL-14 ELISA (Cloud-Clone Corp, Houston, TX, USA) had an assay range of 15.6-1000 pg/ml, a sensitivity of < 5.6 pg/ml, and intra- and inter-assay coefficients of variation of < 10%. The IL-16 ELISA (Thermo Scientific, Inc., Rockford, IL, USA) had an assay range of 0-2000 pg/ml, a

sensitivity of < 8.0 pg/ml, and intra- and inter-assay coefficients of variation of < 10%. In the

ELISAs, mean OD_{450} values from duplicate samples of thyrocyte culture supernatants were

corrected for background using the appropriate 0 h mean OD_{450} value. IL-14 and IL-16

protein levels were then calculated as pg/ml from the appropriate standard curve.

Results

IL-14 and IL-16 mRNA and protein expression in thyroid tissue samples

The expression of IL-14 and IL-16 mRNA was analysed in thyroid tissue using RT-PCR amplification. IL-14 mRNA expression was detected in thyroid tissue from 8/9 GD, 3/4 HT and 3/13 MNG patients, as well as 1/6 normal individuals (Fig. 1a). IL-16 mRNA was found in thyroid tissue from 9/9 GD, 4/4 HT and 9/13 MNG patients, in addition to 4/6 normal individuals (Fig. 1a).

The expression of IL-14 and IL-16 protein was analysed in thyroid tissue extracts using western blotting. IL-14 protein was detected as a 70-kD band in thyroid tissue extracts derived from 6/6 GD and 1/1 HT patients, but not in any samples taken from six MNG patients or six normal individuals (Fig. 2a). This was greater than the expected molecular weight of 60-62 kD.[28] IL-16 protein was identified at 68 kD in thyroid tissue extracts from 6/6 GD, 1/1 HT and 1/6 MNG patients, but was not found in any of six normal individuals (Fig. 2a). The protein detected by western blotting was assumed to represent the precursor form of IL-16, pro-IL-16, which has a molecular weight estimated from its cDNA sequence of 69 kD, in comparison to the secreted 14-kDa active C-terminal part of the cytokine.[29]

IL-14 and IL-16 mRNA expression in intra-thyroidal CD4^{+} and CD8^{+} T cells

As the thyroids of patients with autoimmune thyroid disease are infiltrated with T lymphocytes which could be a source of IL-14 and IL-16 in the tissue, the expression of mRNA for both cytokines was analysed by RT-PCR amplification in intra-thyroidal CD4^{+} and CD8^{+} T cells derived from two GD and two HT patients. IL-14 mRNA was detected in both intra-thyroidal CD4^{+} and CD8^{+} T cells from all four patients (Fig. 1b). In contrast, IL-16 mRNA

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was detected in intra-thyroidal CD4$^+$ and CD8$^+$ T cells derived from only one of the two HT patients (Fig. 1b) and in none of the samples taken from the two GD patients.

IL-14 and IL-16 mRNA and protein expression in thyroid follicular cells

To investigate whether thyroid follicular cells could be a source of IL-14 and IL-16 in the thyroids of autoimmune disease patients, the expression of both cytokines in terms of mRNA and protein was analysed. IL-14 and IL-16 mRNA expression was detected by RT-PCR amplification in cultured HT-ori3 cells and in thyroid follicular cells derived from two patients with GD and one normal individual (Fig. 1c).

By western blotting, IL-14 and IL-16 proteins were not detectable in extracts of cultured HT-ori3 cells or cultured thyroid follicular cells derived from two patients with GD and from one normal individual (Fig. 2b). In contrast, both IL-14 and IL-16 proteins were evident in extracts of all thyrocyte cultures following treatment with PBMC-conditioned medium (Fig. 2b).

Effect of cytokine and TSH treatment on IL-14 and IL-16 protein expression by cultured thyrocytes

To analyse the potential for cytokines and TSH to affect the expression of IL-14 and IL-16 protein by thyrocytes, cultured HT-ori3 cells and thyroid follicular cells derived from two patients with GD and from one normal individual, were treated with IL-1β, IL-1α, IL-17A, IL-4, TGF-β, TNF-α, IFN-γ, PBMC-conditioned medium and TSH. IL-14- and IL-16-specific ELISAs were used subsequently to detect the levels of IL-14 and IL-16 protein expressed and secreted into the culture medium by untreated and treated cultured thyrocytes.

The results showed that PMBC-conditioned medium induced IL-14 protein expression, which was detected after 6 h of stimulation, by HT-ori3 cells, thyrocytes from GD patients, and thyroid follicular cells from a normal individual (Fig. 3a-c). IL-14 protein expression was not detectable by HT-ori3 cells, thyroid follicular cells from GD patients or thyrocytes from a normal individual (Fig. 3a-c) even after treatment for 24 h with IL-1α, IFN-γ, TSH or TNF-α. Likewise, TGF-β, IL-1β, IL-4 and IL-17A were not stimulatory factors for IL-14 protein expression by HT-ori3 cells (Fig. 3a) or by thyrocytes from a normal individual (Fig. 3c).

IL-16 protein expression by all thyrocyte cultures was increased following stimulation with either TNF-α for 24 h or PBMC-conditioned medium for 6 h, when compared with untreated thyroid follicular cells (Fig. 3d-f). In addition, 6-h stimulation of HT-ori3 cells and thyrocytes from a normal individual with IL-1β, IL-17A, IL-4 or TGF-β resulted in increases in IL-16 protein expression when compared with untreated cultures at the same time point (Fig. 3e and f). IL-16 protein expression by all thyrocyte cultures remained undetectable at 24 h without treatment and following stimulation with either IL-1α, IFN-γ or TSH (Fig. 3d-f).

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Discussion

IL-14 is produced by B cells, T cells and dendritic cells, and enhances B cell proliferation, increases the sub-population of memory B cells and prevents the secretion of immunoglobulins.[10] In the present study, novel findings in relation to IL-14 expression in autoimmune thyroid disease patients are reported. IL-14 mRNA expression was detected in the majority of GD and HT patient thyroid tissue samples, but was only evident in a minority of MNG patients and normal individuals. IL-14 protein was found in GD and HT patient thyroid tissue, but not in MNG patients or normal individuals.

Next, the cellular source of IL-14 mRNA and protein in thyroid tissue affected by autoimmune disease was investigated. Thyroid-infiltrating lymphocytes isolated from HT and GD patients expressed IL-14 mRNA, as did cultured thyroid follicular cells derived from patients with GD and normal individuals. However, despite clear expression of IL-14 mRNA in thyroid follicular cells *in vitro*, IL-14 mRNA was detected in thyroid tissue samples from only three of 13 MNG patients and one of six normal individuals even after PCR amplification using a high cycle number. It could be that unknown inhibitory factors operate to suppress *in vivo* IL-14 mRNA expression by thyrocytes in MNG and normal thyroid tissue.

Concerning IL-14 protein expression by thyrocytes, this was apparent only after stimulation with PBMC-conditioned medium. None of the cytokines tested nor TSH induced production of IL-14 protein by cultured thyroid follicular cells, indicating that some novel combination of factors in the conditioned medium is most likely to be responsible. Overall, the data suggest that both suitably stimulated thyroid follicular cells and infiltrating T cells are potential sources of the IL-14 protein that is found in the thyroid tissue of patients with autoimmune thyroid disease. By producing IL-14, both cell types may play a part in driving

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the intra-thyroidal production of thyroid autoantibodies,[30] given the importance of the

cytokine in B cell proliferation, memory B cell production and immunoglobulin synthesis.

IL-16 has a key role in inflammatory and autoimmune processes. It attracts CD4$^+$ T

cells, stimulates resting T cells, and enhances the production of pro-inflammatory cytokines

by immune-response cells.[13] In this study, IL-16 mRNA expression was found in all GD and

HT thyroids and in the thyroid tissue of the majority of MNG patients and normal

individuals. IL-16 protein was detected in thyroid tissue extracts from all of the GD and HT

patients analysed, but was not evident in most MNG patient thyroids and not at all in

normal individuals. These results are consistent with a previous report concerning IL-16

expression in thyroids affected by autoimmunity.[22]

Having detected IL-16 mRNA in the majority of thyroid tissue and protein expression in

diseased thyroids, it was necessary to determine which cells in the thyroid were able to

express this cytokine. IL-16 mRNA expression was not evident in intra-thyroidal CD4$^+$ and

CD8$^+$ T cells derived from the majority of patients with GD or HT, although IL-16 mRNA and

IL-16 protein are constitutively expressed in T lymphocytes from normal individuals.[31]

Expression of IL-16 mRNA was, however, detected in thyroid follicular cells whether derived

from patients with autoimmune or non-autoimmune thyroid conditions or from normal

individuals. This result is analogous to earlier reports in which constitutive IL-16 mRNA

expression by thyrocytes *in vitro* has been shown.[22] Similarly, fibroblasts from normal and

GD thyroids constitutively express IL-16 mRNA.[32]

With regard to IL-16 protein expression in thyrocytes, earlier studies reported that this

did not occur in unstimulated cultured thyroid follicular cells and could only be detected

following treatment of cultures with either IL-1β, TNF-α, IL-4 or TGF-β, although not by IFN-γ

nor TSH.[22] These observations were confirmed in the present study. There have also been

similar discoveries with respect to thyroid-derived fibroblasts from patients with GD: expression of IL-16 protein is stimulated by IL-1β and TNF-α.[32] The finding that IL-16 protein expression in cultured thyrocytes can be induced by IL-17A is novel, although this pro-inflammatory cytokine does increase markedly the production of IL-16 in synovial fibroblasts from patients with rheumatoid arthritis.[33] Interestingly, IL-16 synthesis can be induced in cultured thyroid follicular cells by GD patient IgG and this is suggested to signal via the insulin-like growth factor-1 receptor, not the thyrotropin receptor, autoimmunity against which is the basis for the development of GD.[23] Indeed, we found no evidence that TSH-stimulation of the thyrotropin receptor caused the production of IL-16 in cultured thyrocytes.

Notably, the expression of IL-16 protein by thyrocyte cultures following cytokine-stimulation showed the same pattern whether from the thyroid of patients with GD or normal individuals. However, the detection of IL-16 protein in the thyroid tissue of GD and HT patients, but not in normal individuals, would suggest there is increased expression of this cytokine by thyrocytes in diseased *versus* normal thyroid glands. This may result from inflammatory factors present in HT and GD thyroids which drive the expression of IL-16 protein by thyroid follicular cells. As a source of IL-16, thyrocytes may play a role, along with thyroidal fibroblasts,[32] in CD4^{+} cell recruitment to and activation within the thyroid gland.

In summary, the current study demonstrates that IL-14 and IL-16 are expressed in GD and HT thyroid tissue, suggesting an involvement of these cytokines in disease pathogenesis. Furthermore, thyroid follicular cells are able to produce IL-14 and IL-16, emphasising a potential role for these cells in promoting thyroid autoimmunity.

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Conflicts of interest

None to declare

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3   **Figure Legends**
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8   **Fig. 1.** IL-14 and IL-16 mRNA expression in thyroid tissue, intra-thyroidal lymphocytes and
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10   cultured thyrocytes. The results of RT-PCR amplification are shown for: **(a)** thyroid tissue
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12   samples from each of four patients with GD, HT or MNG and from four normal individuals;
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15   **(b)** intra-thyroidal CD4$^+$ and CD8$^+$ T cells derived from a patient with GD and a patient with
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17   HT; and **(c)** cultured HT-ori3 cells and cultured thyroid follicular cells derived from a patient
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20   with GD and a normal individual. PCR amplification products were identified at 319, 399 and
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22   560 base-pairs for IL-14, IL-16, and β-actin, respectively.
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27   **Fig. 2.** IL-14 and IL-16 protein expression in thyroid tissue and cultured thyrocytes. The
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29   results of western blotting are shown for: **(a)** thyroid tissue samples from a patient with
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31   either GD, HT or MNG and from a normal individual; and **(b)** cultured HT-ori3 (O) cells, and
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34   cultured thyroid follicular cells derived from a patient with GD and a normal (N) individual
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36   both untreated and treated with PBMC-conditioned medium for 24 h. IL-14 and IL-16
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39   proteins were identified at 70 kD and 68 kD, respectively.
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43   **Fig. 3.** Effect of treatment with cytokines, PMBC-conditioned medium and TSH on the
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46   expression of IL-14 and IL-16 protein by cultured thyrocytes. The results of IL-14- and IL-16-
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48   specific ELISAs with IL-14 and IL-16 levels expressed as pg/ml (mean ± SD of two
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50   experiments) are shown, respectively, for: **(a and d)** HT-ori3 cells; **(b and e)** thyroid follicular
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53   cells from a GD patient; and **(c and f)** thyroid follicular cells from a normal individual.
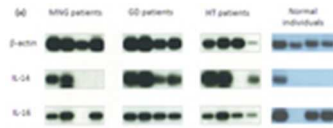54
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Fig. 1. IL-14 and IL-16 mRNA expression in thyroid tissue, intra-thyroidal lymphocytes and cultured thyrocytes. The results of RT-PCR amplification are shown for: (a) thyroid tissue samples from each of four patients with GD, HT or MNG and from four normal individuals; (b) intra-thyroidal CD4+ and CD8+ T cells derived from a patient with GD and a patient with HT; and (c) cultured HT-ori3 cells and cultured thyroid follicular cells derived from a patient with GD and a normal individual. PCR amplification products were identified at 319, 399 and 560 base-pairs for IL-14, IL-16, and ββ-actin, respectively.
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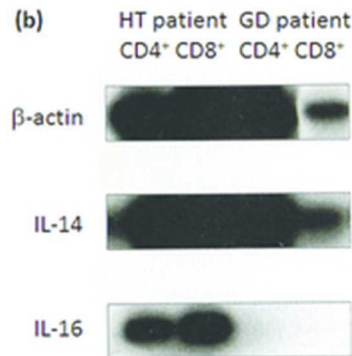49
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Fig. 1. IL-14 and IL-16 mRNA expression in thyroid tissue, intra-thyroidal lymphocytes and cultured thyrocytes. The results of RT-PCR amplification are shown for: (a) thyroid tissue samples from each of four patients with GD, HT or MNG and from four normal individuals; (b) intra-thyroidal CD4+ and CD8+ T cells derived from a patient with GD and a patient with HT; and (c) cultured HT-ori3 cells and cultured thyroid follicular cells derived from a patient with GD and a normal individual. PCR amplification products were identified at 319, 399 and 560 base-pairs for IL-14, IL-16, and ββ-actin, respectively.
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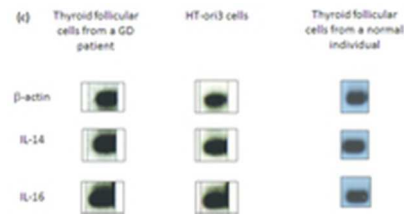51
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Fig. 1. IL-14 and IL-16 mRNA expression in thyroid tissue, intra-thyroidal lymphocytes and cultured thyrocytes. The results of RT-PCR amplification are shown for: (a) thyroid tissue samples from each of four patients with GD, HT or MNG and from four normal individuals; (b) intra-thyroidal CD4+ and CD8+ T cells derived from a patient with GD and a patient with HT; and (c) cultured HT-ori3 cells and cultured thyroid follicular cells derived from a patient with GD and a normal individual. PCR amplification products were identified at 319, 399 and 560 base-pairs for IL-14, IL-16, and $\beta\beta$-actin, respectively.
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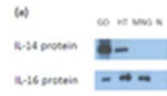48
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Fig. 2. IL-14 and IL-16 protein expression in thyroid tissue and cultured thyrocytes. The results of western blotting are shown for: (a) thyroid tissue samples from a patient with either GD, HT or MNG and from a normal individual; and (b) cultured HT-ori3 (O) cells, and cultured thyroid follicular cells derived from a patient with GD and a normal (N) individual both untreated and treated with PBMC-conditioned medium for 24 h. IL-14 and IL-16 proteins were identified at 70 kD and 68 kD, respectively.
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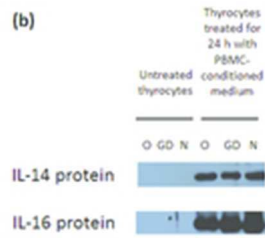47
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Fig. 2. IL-14 and IL-16 protein expression in thyroid tissue and cultured thyrocytes. The results of western blotting are shown for: (a) thyroid tissue samples from a patient with either GD, HT or MNG and from a normal individual; and (b) cultured HT-ori3 (O) cells, and cultured thyroid follicular cells derived from a patient with GD and a normal (N) individual both untreated and treated with PBMC-conditioned medium for 24 h. IL-14 and IL-16 proteins were identified at 70 kD and 68 kD, respectively.
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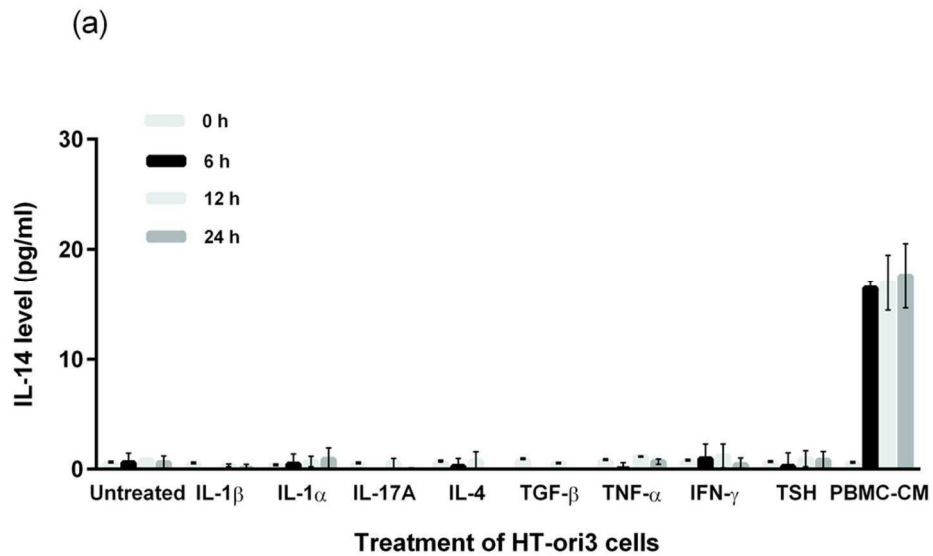46
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Fig. 3. Effect of treatment with cytokines, PMBC-conditioned medium and TSH on the expression of IL-14 and IL-16 protein by cultured thyrocytes. The results of IL-14- and IL-16-specific ELISAs with IL-14 and IL-16 levels expressed as pg/ml (mean ± SD of two experiments) are shown, respectively, for: (a and d) HT-ori3 cells; (b and e) thyroid follicular cells from a GD patient; and (c and f) thyroid follicular cells from a normal individual.
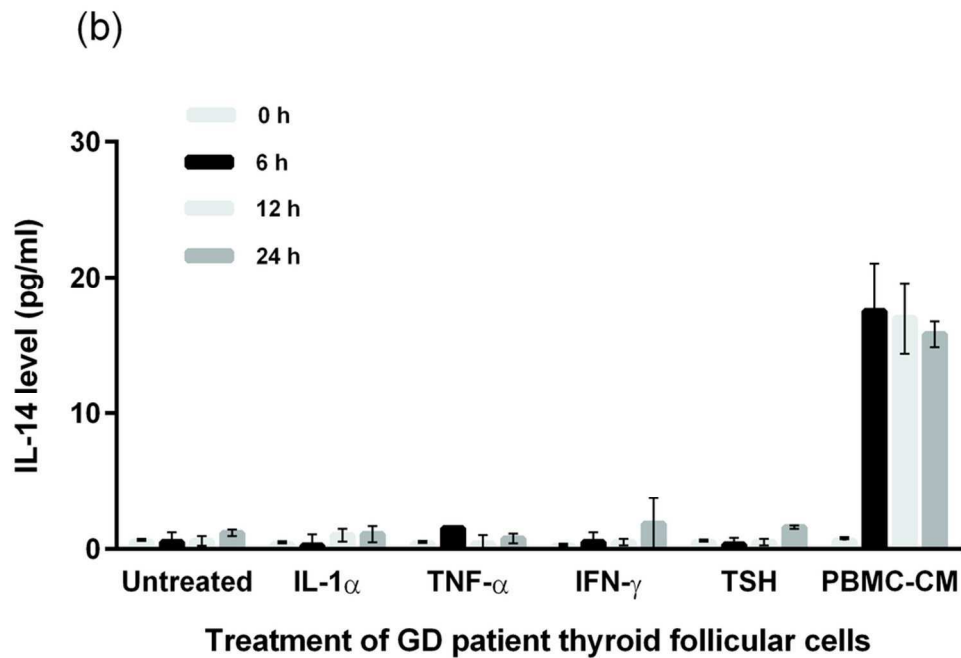92x55mm (300 x 300 DPI)

Fig. 3. Effect of treatment with cytokines, PMBC-conditioned medium and TSH on the expression of IL-14 and IL-16 protein by cultured thyrocytes. The results of IL-14- and IL-16-specific ELISAs with IL-14 and IL-16 levels expressed as pg/ml (mean ± SD of two experiments) are shown, respectively, for: (a and d) HT-ori3 cells; (b and e) thyroid follicular cells from a GD patient; and (c and f) thyroid follicular cells from a normal individual.
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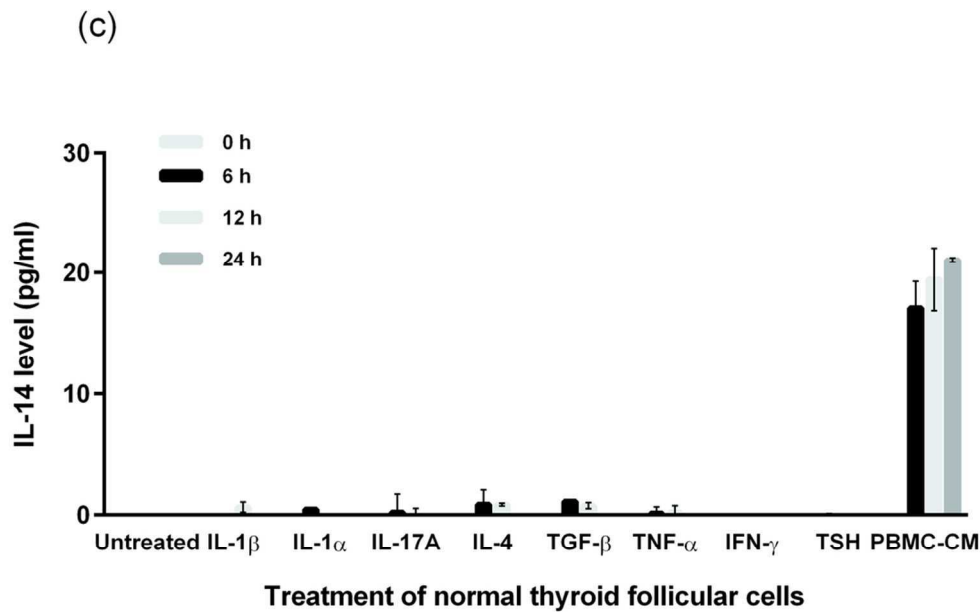44
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(c)

Fig. 3. Effect of treatment with cytokines, PMBC-conditioned medium and TSH on the expression of IL-14 and IL-16 protein by cultured thyrocytes. The results of IL-14- and IL-16-specific ELISAs with IL-14 and IL-16 levels expressed as pg/ml (mean ± SD of two experiments) are shown, respectively, for: (a and d) HT-ori3 cells; (b and e) thyroid follicular cells from a GD patient; and (c and f) thyroid follicular cells from a normal individual.
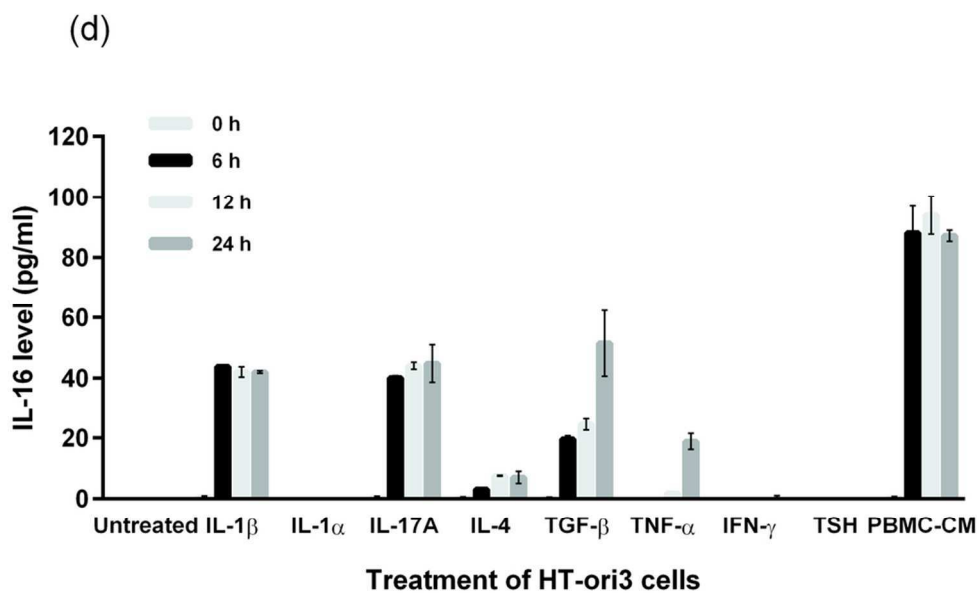92x61mm (300 x 300 DPI)

Fig. 3. Effect of treatment with cytokines, PMBC-conditioned medium and TSH on the expression of IL-14 and IL-16 protein by cultured thyrocytes. The results of IL-14- and IL-16-specific ELISAs with IL-14 and IL-16 levels expressed as pg/ml (mean ± SD of two experiments) are shown, respectively, for: (a and d) HT-ori3 cells; (b and e) thyroid follicular cells from a GD patient; and (c and f) thyroid follicular cells from a normal individual.
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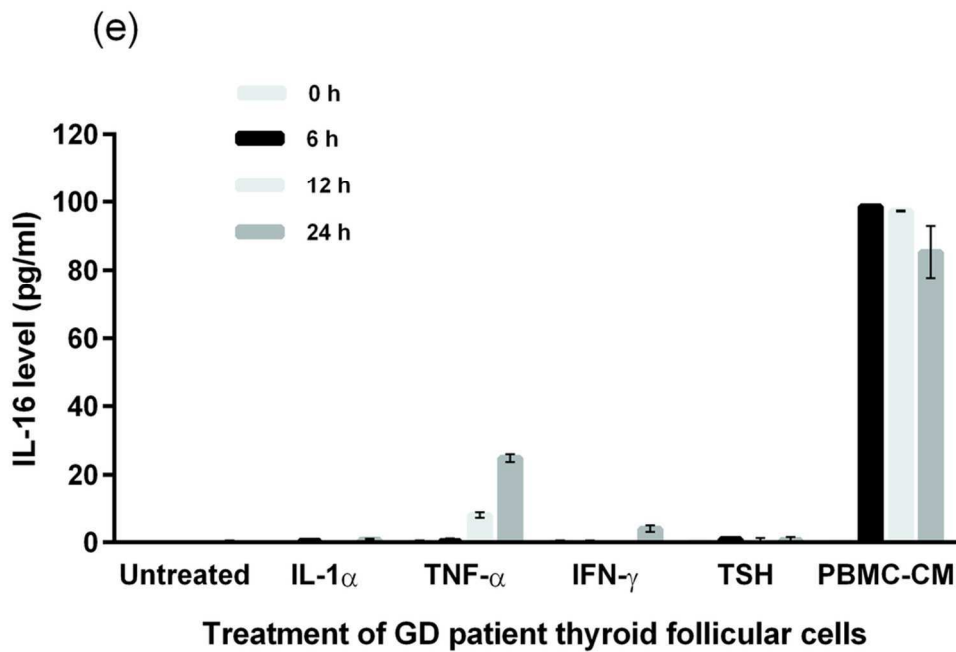44
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Fig. 3. Effect of treatment with cytokines, PMBC-conditioned medium and TSH on the expression of IL-14 and IL-16 protein by cultured thyrocytes. The results of IL-14- and IL-16-specific ELISAs with IL-14 and IL-16 levels expressed as pg/ml (mean ± SD of two experiments) are shown, respectively, for: (a and d) HT-ori3 cells; (b and e) thyroid follicular cells from a GD patient; and (c and f) thyroid follicular cells from a normal individual.
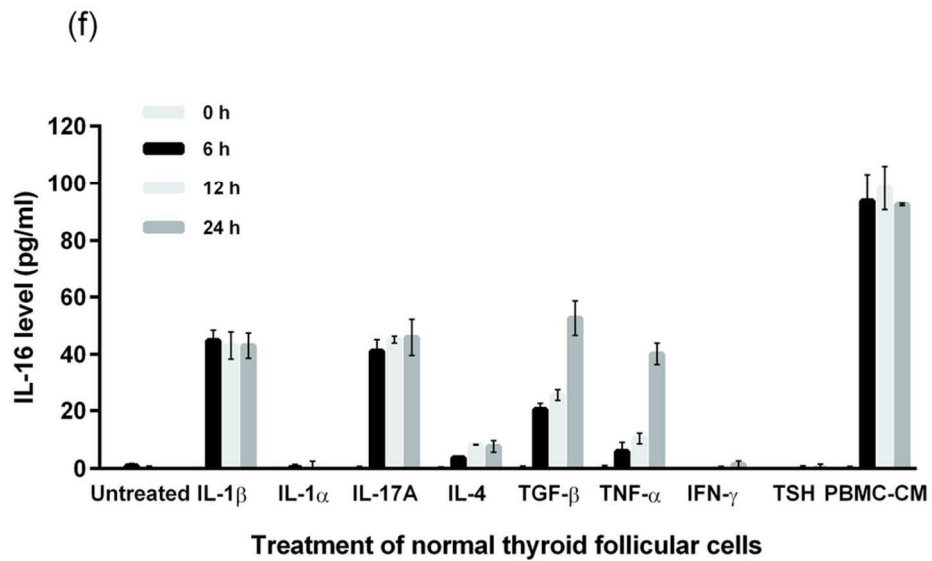89x64mm (300 x 300 DPI)

Fig. 3. Effect of treatment with cytokines, PMBC-conditioned medium and TSH on the expression of IL-14 and IL-16 protein by cultured thyrocytes. The results of IL-14- and IL-16-specific ELISAs with IL-14 and IL-16 levels expressed as pg/ml (mean ± SD of two experiments) are shown, respectively, for: (a and d) HT-ori3 cells; (b and e) thyroid follicular cells from a GD patient; and (c and f) thyroid follicular cells from a normal individual.
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