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Modulation of Fas-mediated apoptosis of human thyroid epithelial cells by IgG from patients with Graves' disease (GD) and idiopathic myxoedema

A. KAWAKAMI, K. EGUCHI, N. MATSUOKA, M. TSUBOI, S. URAYAMA, Y. KAWABE, K. TAHARA*, N. ISHIKAWA†, K. ITO† & S. NAGATAKI *First Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, *Second Department of Internal Medicine, School of Medicine, Chiba University, Chiba, and †Ito Hospital, Tokyo, Japan*

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SUMMARY

The expression of two autoimmune thyroid diseases, GD and idiopathic myxoedema, is associated with antibodies to the thyroid-stimulating hormone (TSH) receptor. Thyroid stimulating antibodies (TSAb) in GD are TSH agonists and cause hyperthyroidism as well as goitre, whereas thyroid stimulation blocking antibodies (TSBAb) in idiopathic myxoedema are TSH antagonists and cause hypothyroidism and thyroid atrophy. We investigated the effect of antibodies to TSH receptor on Fas-mediated apoptosis of thyroid epithelial cells (thyrocytes). Human IgG was isolated from healthy donors, patients with GD and idiopathic myxoedema. Human thyrocytes were obtained from surgical specimens. Thyrocytes were cultured in the presence or absence of human IgG with or without interferon-gamma (IFN-γ) or IL-1β for a specified time. After incubation, we examined the level of cAMP in cultured supernatants and both Fas and Bcl-2 expression on thyrocytes. In addition, we examined anti-Fas-mediated apoptosis of thyrocytes. Fas expression on thyrocytes was significantly down-regulated by Graves' IgG and TSH, although idiopathic myxoedema IgG did not affect Fas expression on thyrocytes. Idiopathic myxoedema IgG abrogated the effect of TSH on both cAMP production and inhibition of Fas expression on thyrocytes. Treatment of thyrocytes with IL-1β or IFN-γ caused a marked augmentation of Fas expression on thyrocytes. The increase of Fas expression of thyrocytes induced by IL-1β or IFN-γ was significantly suppressed in the presence of TSH or Graves' IgG. Anti-Fas-induced apoptosis of thyrocytes was observed in thyrocytes treated with IL-1β or IFN-γ, but was markedly inhibited in the presence of TSH or Graves' IgG. Furthermore, idiopathic myxoedema IgG abrogated most of the inhibitory effect of TSH on Fas-mediated apoptosis of thyrocytes treated with IL-1β or IFN-γ. Bcl-2 expression of thyrocytes did not change after stimulation with TSH, Graves' IgG, idiopathic myxoedema IgG, IL-1β or IFN-γ. These results suggest that TSAb found in Graves' patients may be potentially involved in the development of goitre by inhibition of Fas-mediated apoptosis of thyrocytes. In addition, TSBAb inhibit the action of TSH and increase the sensitivity toward Fas-mediated apoptosis of thyrocytes, inducing thyroid atrophy seen in patients with idiopathic myxoedema.

Keywords thyroid-stimulating antibodies thyroid stimulation blocking antibodies Fas-mediated apoptosis thyrocytes Bcl-2

INTRODUCTION

Programmed cell death, or apoptosis, is a physiological suicide mechanism for maintaining cellular homeostasis in a variety of tissues [1]. A high rate of apoptosis is present in endocrine tissues following hormone deprivation. For example, castration in rats induces apoptosis of prostate cells [2,3], and canine thyrocytes undergo apoptosis following deprivation of thyroid-stimulating hormone (TSH) [4]. These results suggest that apoptosis of endocrine cells is suppressed by their own specific hormones and that the regulation of apoptosis may be critical in endocrine tissue homeostasis.

Previous reports showed that apoptosis is tightly controlled by various gene products [5]. Among these, Fas has been identified as the putative molecule capable of transducing apoptotic signals into cells [6]. We have recently reported that Fas is functionally expressed on thyrocytes and that anti-Fas-induced apoptosis of

Correspondence: Atsushi Kawakami MD, The First Department of Internal Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852, Japan.

thyrocytes is significantly suppressed by treating cells with TSH [7]. Our data indicated that the growth-promoting activity of TSH on the thyroid glands may, in part, be achieved by the inhibitory effect of Fas-mediated apoptosis of thyrocytes.

Recent studies have shown that anti-TSH receptor autoantibodies are involved in the pathogenesis of GD and idiopathic myxoedema, although the precise mechanism is still not well understood [8–14]. Thyroid-stimulating antibodies (TSAb) found in patients with GD are competitive agonists of TSH and induce hyperthyroidism as well as goitre [8–14]. In contrast, thyroid stimulation blocking antibodies (TSBAb) in patients with idiopathic myxoedema inhibit TSH binding and cause hypothyroidism and thyroid atrophy [8,9].

In the present study, we examine the modulatory effects of immunoglobulins derived from patients with autoimmune thyroid diseases on Fas-mediated apoptosis of human thyrocytes.

MATERIALS AND METHODS

Reagents

Reagents used in the present studies included recombinant interferon-gamma (rIFN-γ; Shionogi Co., Osaka, Japan) and rIL-1β (Otsuka Pharmaceutical Co., Tokushima, Japan). Bovine TSH were purchased from Sigma Chemical Co. (St Louis, MO). The murine MoAbs and controls used in the present experiments included anti-CD3 MoAb (Coulter Immunology, Hialeah, FL), anti-CD20 MoAb (Coulter), anti-CD14 MoAb (Coulter), anti-von Willebrand factor MoAb (Immunotech, Marseilles, France), anti-Fas MoAb (IgM and PE-conjugated IgG1; MBL, Nagaya, Japan), FITC-conjugated anti-Bcl-2 MoAb (IgG1; Dako Japan, Kyoto, Japan), control mouse IgG1 (PE-conjugated from MBL, FITC-conjugated from Dako) and control mouse IgM (Seikagaku Co., Tokyo, Japan).

Isolation of human IgG

IgG from healthy donors ($n=5$), patients with GD ($n=6$) or idiopathic myxoedema ($n=2$) was obtained as previously described, after obtaining signed consent from each patient [15]. To highlight the effect of TSAb and TSBAb, we selected patients' sera using three kinds of methods, including TSH receptor antibody (TRAb) assay, TSAb assay, and TSBAb assay, as previously described [16]. In brief, TRAb was measured by radioreceptor assay using ^{125}I (>40% is positive). Both TSAb (>140% is positive) and TSBAb (>40% is positive) were measured by bioassay in response to cAMP production using FRTL5 cells. To mimic the effect of pure TSAb, Graves' patients' sera ($n=6$) contained high TSAb activity without, or with minimum, TSBAb activity (TRAb > 80%, TSAb > 450%, TSBAb < 3%), and to mimic that of TSBAb, idiopathic myxoedema patients' sera ($n=2$) contained high TSBAb activity without TSAb activity (TRAb > 80%, TSAb < 140%, TSBAb > 80%). Briefly, serum was dialysed against 10 mM Tris–HCl buffer pH 7·4 and applied to a protein A-Sepharose column. After eluting with 0·1 M glycine–HCl pH 3·0, the elute was immediately neutralized with 1·0 N NaOH and then extensively dialysed against 0·9% NaCl solution. Finally, the elutes were dialysed against culture medium and sterilized by filtering through a 0·45-μm filter (Millipore Corp., Bedford, MA).

Preparation of thyrocytes

Thyroid tissue samples were obtained from patients with GD ($n=12$), all of whom were treated with an anti-thyroid drug and/or β-adrenergic antagonist drugs, and were euthyroid at the time of subtotal thyroidectomy. In some experiments, normal thyroid tissue adjacent to a malignant tumour ($n=5$) was obtained from surgical specimens. The experimental protocol was approved by the Hospital Human Ethics Review Committee and a signed consent was obtained from each patient. The method used for the isolation of thyrocytes was described previously in detail [7,17]. The resultant thyrocyte preparations were <1% reactive with MoAbs, CD3, CD20, CD14, and anti-von Willebrand factor, which, respectively, define an antigen on all mature T cells, pan-B cells, monocytes/macrophages, and on vascular endothelial cells. Moreover, thyrocyte preparations were >99% reactive with anti-thyroglobulin antibody, as confirmed immunohistologically using the avidin-biotin immunoperoxidase technique.

cAMP production from thyrocytes

Thyrocytes (3×10^5) were cultured in the presence or absence of reagents described above for 48 h in RPMI 1640 supplemented with 2% bovine serum albumin (BSA; Sigma) in a six-well tissue culture cluster (Costar, Cambridge, MA). After incubation, cultured supernatants were collected, filtered through a 0·45-μm filter (Millipore), and the concentration of cAMP in the cultured supernatants was examined as recommended by the manufacturer (radioimmunoassay; Yamasa Chou Co., Chiba, Japan).

Flow cytometric analysis of Fas and Bcl-2 on thyrocytes

Thyrocytes (3×10^5) were cultured in the presence or absence of the reagents described above for 48 h in RPMI 1640 supplemented with 2% BSA (Sigma) in a six-well tissue culture cluster (Costar). After incubation, thyrocytes were detached from the plate by the addition of 0·265 mM EDTA and the detached cells were washed twice with PBS containing 1% fetal bovine serum (FBS).

Fas expression on thyrocytes was examined using a direct immunofluorescence method with PE-conjugated anti-Fas MoAb on a flow cytometer (EPICS-PROFILE-II; Coulter). To investigate the expression of Bcl-2 on thyrocytes, cells were permeabilized with digitonin as described previously [7,18]. Following confirmation of the adequacy of permeabilization by trypan blue uptake, permeabilized cells were incubated with FITC-conjugated anti-Bcl-2 MoAb and examined by a flow cytometer. To check the adequacy of permeabilization on flow cytometric analysis, we used anti-α-tubulin MoAb (Cedarlane Labs, Ontario, Canada) as positive control antibody [7,18]. More than 99% of cells were positive with anti-α-tubulin MoAb.

Induction of apoptosis of thyrocytes by anti-Fas IgM MoAb

Cultured thyrocytes were examined for anti-Fas IgM-induced apoptosis. Thyrocytes were cultured in RPMI 1640 containing 2% BSA in the presence or absence of various reagents listed above, then treated with anti-Fas IgM (1000 ng/ml) or control IgM (1000 ng/ml) for 18 h as previously described [7]. After detaching the cells from the plate, the cells were fixed with 70% ethanol and treated with 100 μg/ml of RNAse (Sigma), and then stained with 100 μg/ml of propidium iodide (Sigma) for 30 min on ice. Apoptosis was quantified by flow cytometric determination of cells with hypodiploid DNA [19].

Statistical analysis

Data were compared using Student's *t*-test, and $P<0\cdot05$ was selected as the level of significance.

Table 1. Effect of Graves' IgG and idiopathic myxoedema IgG on Fas expression of thyrocytes

	Fas expression	
	Percent of positive cells	Mean fluorescence intensity
Control	49·2 ± 4·0	1·56 ± 0·08
Normal IgG	49·6 ± 4·1	1·59 ± 0·07
Graves IgG		
Pt 1	18·5 ± 1·5*	0·96 ± 0·04*
Pt 2	19·4 ± 1·6*	0·96 ± 0·05*
Pt 3	18·9 ± 2·2*	0·92 ± 0·05*
Pt 4	19·9 ± 2·9*	0·95 ± 0·04*
Pt 5	20·5 ± 2·8*	0·95 ± 0·05*
Pt 6	20·9 ± 2·7*	0·93 ± 0·04*
Myxoedema IgG		
Pt 1	47·8 ± 3·9	1·61 ± 0·09
Pt 2	50·4 ± 3·7	1·63 ± 0·09
TSH	17·2 ± 2·5*	0·94 ± 0·05*

Thyrocytes from patients with GD were treated with 5 mg/ml of Graves' IgG ($n = 6$), idiopathic myxoedema IgG ($n = 2$), or 5 mU/ml of thyroid-stimulating hormone (TSH) for 48 h. After incubation, we examined Fas expression on thyrocytes, as described in the text. Data are expressed as mean ± s.d. of six experiments.

*$P < 0{\cdot}01$, compared with control or normal IgG. Control, Thyrocytes treated without IgG (medium only). PE-conjugated isotype-matched control (MBL): 0·7 ± 0·009% and 0·86 ± 0·06.

RESULTS

Effect of Graves' IgG and idiopathic myxoedema IgG on Fas and Bcl-2 expression of thyrocytes

We have recently reported that treatment of thyrocytes with TSH produced a significant inhibition of Fas expression on thyrocytes, and the maximum effect was observed after 48 h incubation [7]. Thus, as the first step, we treated thyrocytes of patients with GD with Graves' IgG for 48 h and then examined Fas expression of these cells by flow cytometry. The treatment of thyrocytes with as little as 1 mg/ml of Graves' IgG (patients 1, 2 and 3) down-regulated Fas expression of thyrocytes, and a more significant effect was observed when the cells were cultured with 5 mg/ml of IgG (data not shown). Therefore, we used control IgG, Graves' IgG, and idiopathic myxoedema IgG at concentrations of 5 mg/ml in the following experiments.

In the next step, we examined the serial effects of Graves' IgG and idiopathic myxoedema IgG on Fas expression of thyrocytes from patients with GD. As shown in Table 1, although Graves' IgG caused a significant suppression of Fas expression on thyrocytes, idiopathic myxoedema IgG did not influence Fas expression on thyrocytes. The mean fluorescence intensity (MFI) of Fas expression on thyrocytes was also down-regulated by Graves' IgG (Table 1) and the level of cAMP in the cultured supernatants increased significantly (data not shown). The viability of thyrocytes, as determined by trypan blue uptake, did not change when cultured with control IgG, Graves' IgG, or idiopathic myxoedema IgG (data not shown).

We previously showed that TSH caused a significant inhibition of Fas expression on thyrocytes without changing Bcl-2 expression

Table 2. Bcl-2 expression on thyrocytes treated with Graves' IgG and idiopathic myxoedema IgG

	Bcl-2 expression	
	Percent of positive cells	Mean fluorescence intensity
Control	95·6 ± 2·2	6·82 ± 0·08
Normal IgG	96·2 ± 2·8	6·86 ± 0·09
Graves IgG		
Pt 1	95·6 ± 2·7	6·85 ± 0·06
Pt 2	96·9 ± 3·3	6·82 ± 0·07
Pt 3	95·7 ± 3·7	6·90 ± 0·08
Pt 4	96·0 ± 2·5	6·83 ± 0·07
Pt 5	95·9 ± 3·5	6·90 ± 0·09
Pt 6	96·2 ± 3·6	6·84 ± 0·08
Myxoedema IgG		
Pt 1	96·5 ± 3·3	6·91 ± 0·08
Pt 2	96·0 ± 2·9	6·91 ± 0·07
TSH	95·5 ± 2·5	6·86 ± 0·07

Thyrocytes from patients with GD were treated with 5 mg/ml of Graves' IgG ($n = 6$), idiopathic myxoedema IgG ($n = 2$) or 5 mU/ml of thyroid-stimulating hormone (TSH) for 48 h. After incubation, we examined Bcl-2 expression on thyrocytes as described in the text. Data are expressed as mean ± s.d. of six experiments. Control, thyrocytes treated without IgG (medium only); FITC-conjugated isotype-matched control, 0·8 ± 0·009% and 0·99 ± 0·09.

Table 3. Inhibitory effect of Graves' IgG on IFN-γ- and IL-1β-induced Fas expression on thyrocytes

	Fas expression	
	Percent of positive cells	Mean fluorescence intensity
IFN-γ (−)	48·4 ± 2·8	1·62 ± 0·07
IFN-γ (+):		
Control	93·0 ± 2·9	3·16 ± 0·11
Normal IgG	93·5 ± 3·5	3·19 ± 0·09
Graves' IgG		
Pt 1	66·8 ± 4·8*	2·34 ± 0·08*
Pt 2	64·0 ± 4·2*	2·30 ± 0·07*
Pt 3	66·0 ± 5·0*	2·33 ± 0·08*
Pt 4	64·9 ± 4·3*	2·31 ± 0·08*
TSH	64·8 ± 4·9*	2·32 ± 0·09*
IL-1β (−)	48·2 ± 3·1	1·62 ± 0·07
IL-1β (+):		
Control	91·8 ± 2·7	3·01 ± 0·10
Normal IgG	90·0 ± 2·7	2·97 ± 0·10
Graves' IgG		
Pt 1	63·8 ± 4·7*	2·25 ± 0·10*
Pt 2	62·1 ± 4·3*	2·24 ± 0·09*
Pt 3	63·0 ± 4·9*	2·19 ± 0·08*
Pt 4	63·9 ± 4·4*	2·21 ± 0·09*
TSH	61·8 ± 4·8*	2·22 ± 0·11*

Thyrocytes from patients with GD ($n = 6$) were treated with IFN-γ (500 U/ml) or IL-1β (10 U/ml) in the presence of 5 mg/ml of control IgG, Graves' IgG ($n = 4$) or 5 mU/ml of thyroid-stimulating hormone (TSH) for 48 h. After incubation, we examined Fas expression on thyrocytes as described in the text.

*$P < 0{\cdot}01$, compared with Fas expression of thyrocytes stimulated by IFN-γ or IL-1β with normal IgG or medium only.

Control, thyrocytes treated without IgG (medium only).

Table 4. Blocking activity of idiopathic myxoedema IgG on the effect of thyroid-stimulating hormone (TSH) on thyrocytes

	TSH	Myxoedema IgG (Pt1)	cAMP (pmol/ml)	Fas expression (%)	Myxoedema IgG (Pt2)	cAMP (pmol/ml)	Fas expression (%)
Experiment 1	−	−	5·74	56	−	8·82	54
	−	+	5·20	54	+	8·90	55
	+	−	128·0	16	−	145·5	18
	+	+	9·50	51	+	12·45	52
Experiment 2	−	−	7·50	52	−	9·10	55
	−	+	5·80	56	+	8·98	54
	+	−	155·5	18	−	110·9	19
	+	+	10·52	48	+	13·50	53

Thyrocytes from patients with GD were treated in the presence of 5 mU/ml of TSH with or without 5 mg/ml of idiopathic myxoedema IgG for 48 h. After incubation, we examined Fas expression on thyrocytes and cAMP level in the cultured supernatant as described in the text. Data shown are the results of two experiments.

[7]. Thus, we examined the effect of Graves' IgG on Bcl-2 expression of thyrocytes. Most thyrocytes from patients with GD expressed Bcl-2, and neither Graves' IgG nor idiopathic myxoedema IgG changed Bcl-2 expression of thyrocytes (Table 2). The effect of Graves' IgG and idiopathic myxoedema IgG on Fas and Bcl-2 expression on normal thyrocytes was almost similar to that on thyrocytes from patients with GD, and we did not find statistical difference in two groups of thyrocytes (data not shown).

Inhibitory effect of Graves' IgG on Fas expression of thyrocytes induced by IFN-γ or IL-1β

We next investigated whether Graves' IgG could suppress IFN-γ- or IL-1β-induced Fas expression on thyrocytes, since our previous work showed that TSH causes a significant inhibition of IFN-γ- or IL-1β-induced Fas expression on thyrocytes [7]. Thyrocytes from Graves' patients were cultured with IFN-γ or IL-1β in the presence of either control IgG or Graves' IgG for 48 h, followed by examination of Fas expression on thyrocytes. As shown in Table 3, Graves' IgG caused a significant inhibition of IFN-γ- or IL-1β-induced Fas expression on thyrocytes, although there was no difference in Bcl-2 expression between these cells and unstimulated thyrocytes (data not shown). In addition, idiopathic myxoedema IgG itself did not affect

Table 5. The inhibitory effect of Graves' IgG on Fas-mediated apoptosis of thyrocytes induced by IFN-γ

	Hypodiploid DNA (%)
IFN-γ (−)	1·2 ± 0·.5
IFN-γ (+)	
Control	39·0 ± 3·9
Normal IgG	38·5 ± 2·9
Graves' IgG	
Pt 1	19·0 ± 3·3*
Pt 2	18·4 ± 2·5*
Pt 3	18·8 ± 2·9*
Pt 4	17·8 ± 2·5*
TSH	18·2 ± 2·9*

Thyrocytes from patients with GD were cultured with IFN-γ (500 U/ml) in the presence or absence of 5 mg/ml of Graves' IgG ($n = 4$) or 5 mU/ml of thyroid-stimulating hormone (TSH) for 48 h. After incubation, the percentage of the cells with hypodiploid DNA was examined as described in the text. Control, thyrocytes treated without IgG (medium only). Data are the mean ± s.d. of the percentage of cells with hypodiploid DNA from five experiments.

*$P < 0{\cdot}01$ compared with control or normal IgG.

Table 6. Blocking activity of idiopathic myxoedema IgG for the inhibitory effect of thyroid-stimulating hormone (TSH) on Fas-mediated apoptosis of thyrocytes treated by IFN-γ

	Hypodiploid DNA (%)
IFN-γ (−)	1·5 ± 0·4
IFN-γ (+)	
Control	40·5 ± 4·6
Normal IgG	39·9 ± 4·6
TSH	18·4 ± 2·1
TSH + normal IgG	19·2 ± 2·5
Myxoedema IgG	
Pt 1	42·2 ± 4·0
Pt 2	43·2 ± 4·9
TSH + myxoedema IgG	
Pt1	36·9 ± 4·5*
Pt2	37·4 ± 4·8*

Thyrocytes from patients with GD were cultured with IFN-γ (500 U/ml) in the presence of reagents listed in Table 6 (5 mg/ml of normal and idiopathic myxoedema IgG, 5 mU/ml of TSH) for 48 h. After incubation, the percentage of the cells with hypodiploid DNA was examined as described in the text. Control, thyrocytes treated without IgG (medium only). Data are the mean ± s.d. of the percentage of cells with hypodiploid DNA from six experiments.

*$P < 0{\cdot}01$ compared with TSH or TSH + normal IgG and not significantly different from control or normal IgG.

Fas expression of thyrocytes induced by IFN-γ or IL-1β (data not shown).

Blocking activity of idiopathic myxoedema IgG on the effect of TSH on thyrocytes

The inhibitory effect of TSH on Fas expression on thyrocytes may be mediated by cAMP [7], while TSBAb found in idiopathic myxoedema inhibit TSH-increased cAMP level [8,9]. Accordingly, we examined the effect of idiopathic myxoedema IgG on Fas expression of thyrocytes inhibited by TSH. As shown in Table 4, TSH-induced inhibition of Fas expression on thyrocytes from Graves' patients recovered to a large extent in the presence of idiopathic myxoedema IgG. In addition, the level of cAMP in the culture supernatant, induced by TSH, was clearly suppressed by the addition of idiopathic myxoedema IgG, suggesting the importance of cAMP for the inhibitory effect of TSH on Fas expression. The effect of idiopathic myxoedema IgG on normal thyrocytes was almost similar to thyrocytes from patients with GD (data not shown).

Inhibitory effect of Graves' IgG on apoptosis of thyrocytes induced by anti-Fas IgM

We examined the effects of Graves' IgG on anti-Fas IgM-induced apoptosis of thyrocytes. Only IFN-γ- or IL-1β-stimulated thyrocytes undergo apoptosis when treated with anti-Fas IgM [7]. Thyrocytes were cultured with control IgG or Graves' IgG for 48 h in the presence of IFN-γ or IL-1β, followed by further incubation with anti-Fas IgM for 18 h. After incubation, apoptotic cell death of thyrocytes was determined by flow cytometry. As shown in Table 5, apoptosis of thyrocytes from patients with GD cultured with IFN-γ in the presence of Graves' IgG was significantly suppressed compared with cells cultured with IFN-γ and control IgG. Anti-Fas IgM-induced apoptosis of thyrocytes cultured with IL-1β was also significantly inhibited by Graves' IgG (data not shown).

Blocking activity of idiopathic myxoedema IgG for the inhibitory effect of TSH on Fas-mediated apoptosis of thyrocytes treated by IFN-γ and IL-1β

We recently reported that TSH significantly suppressed Fas-mediated apoptosis of thyrocytes treated by IFN-γ or IL-β [7]. Since idiopathic myxoedema IgG blocked the effect of TSH (Table 4), we finally investigated the blocking activity of idiopathic myxoedema IgG for the inhibitory effect of TSH on Fas-mediated apoptosis of thyrocytes treated by IFN-γ or IL-1β. As shown in Table 6, idiopathic myxoedema IgG abrogated most of the inhibitory effect of TSH on Fas-mediated apoptosis of thyrocytes from patients with GD induced by IFN-γ.

The same results were obtained when IL-1β-stimulated thyrocytes were used in these sets of experiments (data not shown). In the final two sets of experiments demonstrating Fas-mediated apoptosis of thyrocytes, we also found no statistical difference in thyrocytes between normal and GD (data not shown).

DISCUSSION

Recent studies have shown the importance of apoptosis in regulating endocrine tissue homeostasis, e.g. prostate cells [20,21] and thyroid glands [22]. Furthermore, Fas is evenly expressed on unstimulated cultured human thyrocytes, and expression of Fas on these cells is significantly augmented by IFN-γ or IL-1β [7]. Anti-Fas-mediated apoptosis of thyrocytes was determined in thyrocytes with markedly increased Fas expression induced by IFN-γ or IL-1β [7]. In the presence of TSH or 8-bromo-cAMP, both Fas expression and anti-Fas-induced apoptosis of thyrocytes were significantly inhibited, suggesting that the growth-promoting activity of TSH on the thyroid gland involves its inhibitory effect on Fas-mediated apoptosis [7].

TSAb found in Graves' patients mimic the action of TSH and cause hyperthyroidism as well as goitre [8–14]. Since TSH inhibits Fas expression on thyrocytes [7], the addition of IgG from patients with GD caused a significant suppression of Fas expression on thyrocytes and increased production of cAMP. In contrast, the addition of idiopathic myxoedema IgG did not affect Fas expression on thyrocytes. On the other hand, both cAMP production and down-regulation of Fas expression on thyrocytes induced by TSH were inhibited with idiopathic myxoedema IgG. Since the addition of cAMP also mimics the inhibitory action of TSH on Fas expression on thyrocytes [7], our data suggest that the effect of Graves' IgG and idiopathic myxoedema IgG on Fas expression is probably mediated by intracellular cAMP level.

Studies from different laboratories have shown conflicting results regarding the role of bcl-2 in anti-Fas-mediated apoptosis. For example, Itoh *et al*. demonstrated that bcl-2 transfectants became resistant to Fas-mediated apoptosis [23]. On the other hand, other reports suggest that the sensitivity of Fas-mediated apoptosis does not correlate with the level of Bcl-2 [24,25]. Colombel *et al*. recently demonstrated *in vivo* increased expression of Bcl-2 in hormone refractory human prostate cancers [26]. Since most unstimulated thyrocytes expressed Bcl-2, we examined whether the expression of Bcl-2 was changed after several kinds of stimulation. The present results show that anti-Fas-mediated apoptosis of thyrocytes treated with IFN-γ or IL-1β was significantly inhibited by Graves' IgG or TSH without a change in Bcl-2 expression. Because both Graves' IgG and TSH down-regulated Fas expression on thyrocytes without a change in Bcl-2 expression, our results indicate the importance of the magnitude of Fas expression in the induction of Fas-mediated apoptosis of human thyrocytes.

We as well as other laboratories showed previously that activated T cells infiltrate into the thyroid gland in patients with GD [27–29]. Activated T cells *in vitro* express Fas ligand (FasL), a natural ligand for Fas, and efficiently kill Fas^+ target cells [30,31], indicating that the regulation of the interaction of Fas and FasL is important in the development of goitre and hyperthyroidism in patients with GD. Considered together, these results suggest that the goitrogenic activity of TSAb in GD may, in part, be associated with the inhibitory effect on Fas-mediated apoptosis of thyrocytes. Furthermore, IgG from idiopathic myxoedema patients interfered with the inhibitory effect of TSH on both Fas expression and Fas-mediated apoptosis of thyrocytes. In addition to cell-mediated immunity, TSBAb may account for the development of idiopathic myxoedema [32]. We speculate that TSBAb in idiopathic myxoedema patients increases Fas expression and sensitivity of Fas-mediated apoptosis of thyrocytes and induces thyroid atrophy.

In conclusion, we demonstrate the effect of TSH receptor antibodies on Fas-mediated apoptosis of thyrocytes. To our knowledge, this is the first report that human autoantibodies can influence Fas-mediated apoptosis of thyrocytes. The goitrogenic activity of TSAb and the effect of TSBAb on thyroid atrophy may be associated with modulation of Fas-mediated apoptosis of thyrocytes.

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