Soluble Fas, Fas ligand and Bcl-2 in autoimmune thyroid diseases: relation to humoral immune response markers

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Abstract

Purpose: To compare soluble Fas, FasL and Bcl-2 in Graves' disease (GD) and Hashimoto's thyroiditis (HT) to the markers of humoral response: aTPO, aTG and aTSHR.

Material and methods: 5 groups of subjects: 1) 14 patients with GD in euthyreosis on methimazol (euGD); 2) 20 patients with hyperthyroid GD (hrGD); 3) 15 patients with HT in euthyreosis on levothyroxine (euHT); 4) 16 patients with hypothyroid HT (hoHT); 5) 12 healthy volunteers age and sex-matched to group 1-4. Serum concentrations of Fas, FasL, Bcl-2, aTPO and aTG were determined by ELISA and aTSHR by RIA.

Results: Levels of sFas were the highest in hoHT: 8.7 (7.2-9.8) ng/ml as compared to the controls ($p<0.01$) and euHT ($p<0.05$). We found positive correlations between sFas and aTPO in all studied groups ($r=0.25$, $p<0.05$) and between sFas and TSH in HT ($r=0.4$, $p<0.05$). In GD there was a positive correlation between sFasL and aTG ($r=0.5$, $p<0.01$) and negative correlations between sFasL and Fas ($r=-0.39$, $p<0.01$) and between sFasL and period of methimazol administration ($r=-0.32$, $p<0.05$). Levels of sBcl-2 were significantly increased in euHT: 31.0 (13.5-44.1) ng/ml as compared to the controls ($p<0.05$) and euGD ($p<0.05$).

Conclusions: Fas/FasL mediated apoptosis plays an important role in the active stage of the autoimmune process of both Hashimoto's thyroiditis and Graves' disease, however, in Hashimoto's thyroiditis they contribute to irreversible damage of thyreocytes. Early detection of Hashimoto's and Graves' diseases allows for the initiation of the proper treatment that probably leads to the reduction of the autoimmune process intensity.

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Introduction

Autoimmune thyroid diseases (AITD) constitute a group of the most common illnesses caused by autoaggression of the immune system, comprising up to 1.5% of the human population [1]. Pathogenesis of Hashimoto's thyroiditis (HT) and Graves' disease (GD) remains unclear in spite of considerable number of studies. Recently published papers support importance of apoptosis mediated by the interaction between Fas ligand (FasL) and Fas present on the follicular cells surface in cytotoxity, leading to thyrocytes destruction in Hashimoto's thyroiditis [2,3]. There is also some data for thyroid-cell apoptosis in Graves' disease [4,5]. Moreover, evidence from animal models of AITD suggests a role of FasL/Fas dependent cell death in selection of immunocompetent cells in the pathogenic process of HT and GD [6]. Fas (CD95, APO-1) as well as FasL (CD95L, CD178) are members of the Tumor Necrosis Factor (TNF) family and are expressed both on activated T lymphocytes and on thyrocytes of patients with AITD [7]. In autoimmune process combination of inflammatory cytokines: TNFα, interferon γ and interleukin 1β may activate expression of FasL on T cells and may sensitized thyroid follicular cells [8]. FasL/Fas pathway may be regulated at different levels including the expression of the Fas receptor and its cleavage, the inhibition of intracellular death domains and the modulation of Bcl-2, an important anti-apoptotic agent [9]. Role of Bcl-2 in AITD is still being studied and needs to be clarified [10]. Markers of humoral immune response: antithyroperoxidase antibodies (aTPO), antithyroglobulin antibodies (aTG) and antithyroropin receptor antibodies (aTSHR) take important part in clinical assessment of patients with HT and GD as a consequence of well known role of their role in pathogenesis of AITD.

Thus, the aim of the study was to compare soluble Fas, FasL and Bcl-2 in GD and HT in relation to aTPO, aTG and aTSHR

Figure 1. **The median and interquartile ranges of the serum Fas levels in patients with euthyroid Graves' disease (n=14), hyperthyroid Graves' disease (n=20), euthyroid Hashimoto's thyroiditis (n=15), hypothyroid Hashimoto's thyroiditis (n=16) and healthy controls (n=12)**

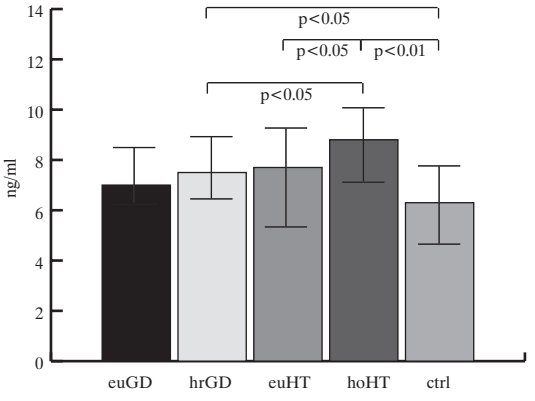
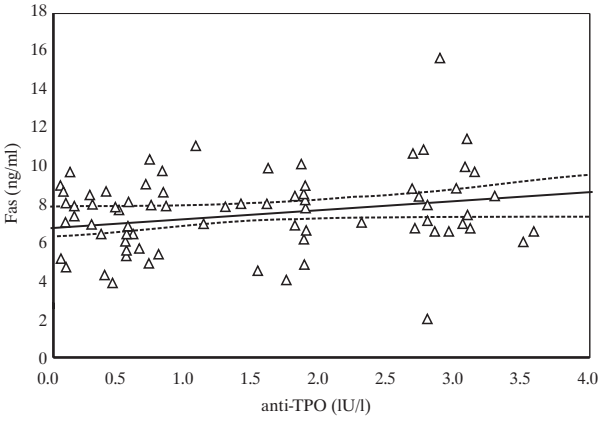


Figure 2. **Positive correlation between serum Fas and aTPO in all the studied individuals (r=0.25, p<0.05)**



to assess a role of proapoptotic Fas/FasL system and antiapoptotic protein Bcl-2 in pathogenesis of AITD.

Material and methods

The study was carried out in 5 groups of subjects: 1) 14 patients with GD in euthyreosis treated with methimazol (euGD): 11 females and 3 males in mean age 41±15 years with a duration of the disease from 7-25 months; 2) 20 patients with hyperthyroid GD (hrGD): 12 females and 8 males aged 42±23 years with a duration of the disease from 3-12 months; 3) 15 patients with HT in euthyreosis treated with levothyroxine (euHT): 15 females in mean age 45±21 years with a duration of the disease from 12-134 months; 4) 16 patients with hypothyroid Ht (hoHT): 14 females and 2 males in mean aged 53±22 years with an diagnosis of the disease 1-12 months prior to the study: and 5) 12 healthy volunteers age and sex-matched to group 1-4 (ctrl): 8 females and 4 males aged 42±18 years who had either no family history of Graves' disease nor other autoimmune diseases. Clinical euthyreosis in groups of 1 and 3 was confirmed by thyrotropin and free thyroxine estimation. Among 77 studied individuals 60 of them (78%) were women to reflect the prevalence relation of AITD in females to males that accounts for 4 -10:1. No acute infections were observed in the studied subjects 3 weeks prior to the study.

All the sera were kept frozen at -70°C until used. The serum levels of Fas, FasL, Bcl-2, aTPO and aTG were determined by the ELISA commercial kits: Fas (Quantikine kit, R&D, Mineapolis, USA; sensitivity 20 pg/ml; intra-assay precision (CV) 3.8%) and FasL (Quantikine kit, R&D, Mineapolis, USA; sensitivity 2.66 pg/ml; CV 4.7%), Bcl-2 (Bender Medsystems, Viena, Austria; sensitivity 0.5 ng/ml; CV 8.6%), aTPO and aTG (DIAMED, Warsaw, Poland; sensitivity respectively 40 IU/ml and 60 IU/ml). The serum levels of aTSHR were determined by the RIA method (TRAK kit, BRAHMS, Berlin, Germany; sensitivity 0.9 IU/L; CV 7.0%). The statistical significance was estimated by Mann-Whitney U-test and to evaluate relationships between variables Spearman's test was performed using Statistica 6.0 for Windows XP (StaSoft, Tulsa, USA).

Results

The results of serum Fas, FasL and Bcl-2 levels in the examined groups are shown in ng/ml as medians and interquartile ranges. Levels of sFas were higher in all the studied groups as compared to the controls, however, the highest values were found in hoHT individuals: 8.7 (7.2-9.8) as compared to the controls: 6.6 (4.4-8.0) ($p<0.01$) and euHT patients: 7.7 (5.2-8.7) ($p<0.05$). There was also a significant difference between hrGD group: 7.6 (6.6-8.5) in comparison with the controls ($p<0.05$) and hoHT patients ($p<0.05$). Relations in sFas values between studied groups are shown in *Fig. 1*.

We found a significant positive correlation between sFas and aTPO in all studied patients ($r=0.25$, $p<0.05$) (*Fig. 2*). There was also a positive correlation between sFas and TSH in HT patients ($r=0.4$, $p<0.05$) (not shown).

There were no significant differences in sFasL concentrations between studied groups: euGD 9.5 (8.5-11.3), hrGD 10.1 (8.4-11.4), euHT 9.7 (8.4-10.3), hoHT 10.7 (7.0-11.3) and the controls 9.7 (8.8-10.9). However, in GD patients we found a positive correlation between sFasL and aTG ($r=0.5$, $p<0.01$) (*Fig. 3*) and negative correlations between sFasL and Fas ($r=-0.39$, $p<0.01$) (*Fig. 4*) and between sFasL and period of methimazol administration ($r=-0.32$, $p<0.05$) (not shown).

Levels of sBcl-2 values were increased in all the AITD groups however significantly higher sBcl-2 values were found only in euHT: 31.0 (13.5-44.1) as compared to the controls: 8.0 (5.0-18.9) ($p<0.05$) and euGD patients: 9.1 (6.6-19.0) ($p<0.05$) (*Fig. 5*).

We found a negative correlation between sBcl-2 and aTPO ($r=-0.38$, $p<0.05$) (*Fig. 6*).

We found no correlation between soluble Fas, FasL and Bcl-2 and thyrotropin and free thyroxine concentration and goiter size.

***Figure 3*. Positive correlation between serum FasL and aTG in GD patients (r=0.52, p<0.01)**

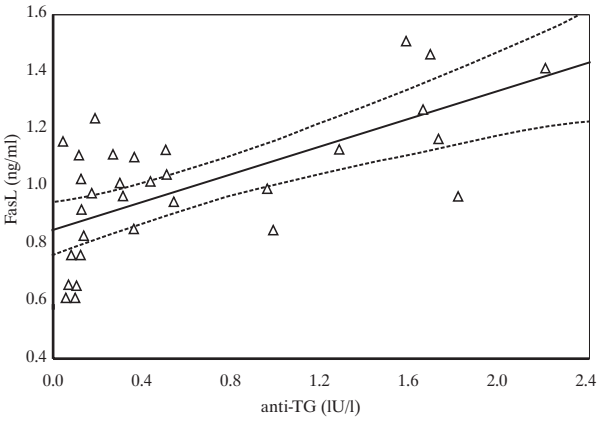


***Figure 4*. Negative correlation between serum FasL and Fas in GD patients (r=-0.39, p<0.01)**

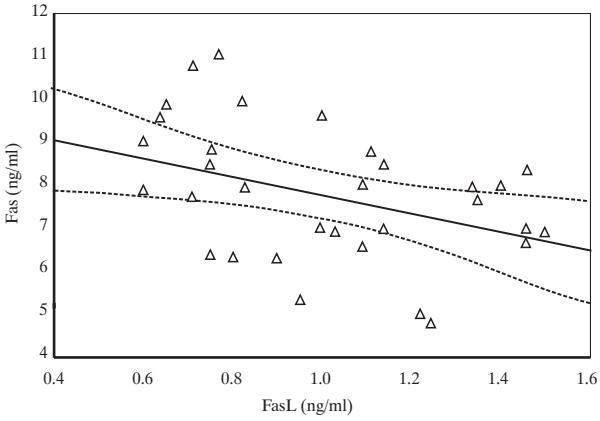


***Figure 5*. The median and interquartile ranges of the serum Bcl-2 levels in patients with euthyroid Graves' disease (n=14), hyperthyroid Graves' disease (n=20), euthyroid Hashimoto's thyroiditis (n=15), hypothyroid Hashimoto's thyroiditis (n=16) and healthy controls (n=12)**

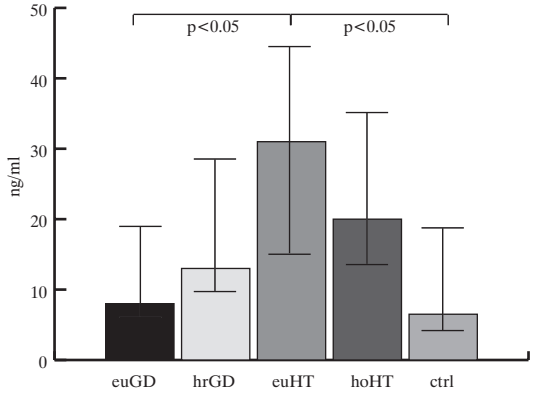
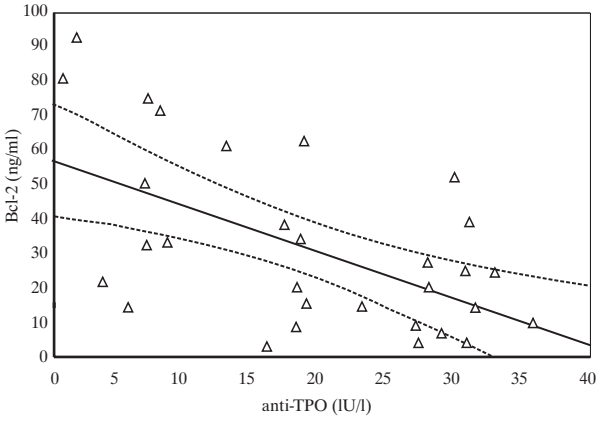


***Figure 6*. Correlation between serum Bcl-2 and aTPO in HT patients (r=-0.38, p<0.05)**



Discussion

Our data have shown increased sFas levels in all the AITD patients, however, the highest concentrations were found in hoHT group. Fas expressed in cell membranes and predisposing to FasL mediated apoptosis may be present both on thyrocytes of patients with AITD and infiltrating lymphocytes. Thus, Fas/ /FasL interaction considerably influences destruction of thyroid tissue on one side and takes important part in the elimination of autoreactive lymphocytes on the other side [10]. Circulating forms of Fas origin both due to the proteolytic cleavage from transmembrane domains and direct mRNA transcription [11]. The results of the recently published studies on the role of soluble Fas and FasL in pathogenesis of AITD remain unequivocal [12,13]. Circulating Fas is commonly considered as a factor that inhibits membrane Fas mediated apoptosis [11]. Our data showing an increase in sFas concentration especially in patients with hypothyroid HT may be a consequence of the enhanced releasing of circulating forms of Fas that seem to reflect the intensity of Fas/FasL mediated apoptosis. As shown by Salmaso et al. in HT apoptosis in a higher degree involves thyrocytes than infiltrating lymphocytes that is connected with a more intense Fas expression on follicular cells [10]. We have found in HT patients positive correlations between TSH and sFas as well as between TSH and aTPO. These findings suggest a relationship between the autoimmune process activity and a degree of hypothyreosis. Moreover, taking into account cytotoxic capacity of aTPO, positve correlation between sFas and aTPO in HT patients suggest that increased sFas may reflect an intensity of the immune involved destruction of the thyroid follicular cells.

Our data on elevated sFas in GD patients is in line with results of Hara et al. which show higher values of circulating Fas in hyperthyroid patients and a positive correlation between sFas and free thyroxine as well as aTSHR [14]. Hiromatsu et al. found a decrease in sFas level in hyperthyroid GD treated with antithyroid drug and a correlation only between sFas and aTSHR, but no correlation between soluble Fas and free triiodothyronine, free thyroxine, thyroid iodine uptake, TSH and aTPO [11]. Antibodies aTSHR are considered an important antiapoptotic factor that may protect thyreocytes of patients with GD from Fas/FasL mediated apoptosis [15]. However,

we did not find any correlation between sFas and aTSHR or thyreometabolic parameters. Our results are in line with data of Takeda et al. [12]. Feldkamp et al. suggest that elevated sFas concentration is a consequence of hyperthyreosis independently on the causes of thyreotoxicosis [16].

Circulating FasL is considered to maintain the capacity to stimulate apoptosis [17]. Taking into account the increased "antiapototic" sFas level in active GD patients, a negative correlation between sFasL and sFas seem to be compliant with findings of a few apoptotic thyrocytes in patients with GD [11]. We have found also a positive correlation between FasL and aTG concentration in GD patints. This data may suggest a role of Fas/FasL dependent apoptosis in autoreactive lymphocyte B selection. Moreover, there was a negative correlation between sFasL and a period of methimazol administration in GD. Antithyroid drugs were shown to manifest immunomodulatory actions. Mitsiades et al. demonstrated the enhanced expression of FasL on thyrocytes in GD patients treated with methimazol [18]. These findings suggest that antithyroid drugs may "arm" thyroid follicular cells with FasL to overcome immunocompetent cells and in consequence to extinguish autoimmune process with accompanied Fas/FasL mediated apoptosis.

Bcl-2 suprafamily proteins were documented to play a crucial role in regulation of apoptosis [18]. Antiapoptotic Bcl-2 expression was shown to be increased in thyrocytes of GD patients in comparison to HT patients and inverse relationship between expression of Bcl-2 on membranes of infiltrating B lymphocytes in GD and HT [11,19]. Moreover, Mitsiades et al. have found thyrocytes in HT to express Bcl-2 less intensively than normal thyroid follicular cells [20]. In spite of numerous studies on membrane Bcl-2 in AITD, data on its circulating form is lacking. We found elevated level of sBcl-2 in HT patients in which euthyreoid state was maintained by levothyroxine. Moreover there was a negative correlation between sBcl-2 and aTPO in HT, suggesting inhibitory influence of Bcl-2 on humoral immune response and thyrocyte destruction, as far as aTPO demonstrates cytotoxic capacity [9]. It may be also speculated that increased sBcl-2 accompanied with decreased sFas level in HT patients in euthyroid state due to levothyroxine may be connected with "protective" influence of exogenes thyroid hormone administration in HT. Slowering of thyrocyte metabolism caused by exogenes levothyroxine may decrease its antigenicity and autoimmune cytotoxic response as it was shown for low-dose insulin in first-degree relatives of type 1 diabetes patients in protection of pancreatic β cells [21].

In summary our results suggest that mechanisms of apoptosis mediated by interaction of Fas and its ligand play an important role in the active stage of the autoimmune process both in pathogenesis of Hashimoto's thyroiditis and Graves' disease, however in Hashimoto's disease it seems to cause irreversible damage of thyreocytes. Early detection of both Hashimoto's thyroiditis and Graves' enables initiation of the proper treatment that probably contributes to the reduction of the autoimmune process intensity.

Acknowledgments

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