BIOPHYSICS AND BIOCHEMISTRY

Increase in Serum Concentration of FAS Ligand as a Possible Mechanism for Antithyroid Cytotoxicity during Autoimmune Thyroid Diseases

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We studied the dependence of serum cytotoxic activity on the contents of soluble apoptosis receptor and its soluble ligand in patients with autoimmune thyroid diseases.

Key Words: *thyroid gland; autoimmune diseases; sFAS; soluble FAS ligand*

Previous studies showed that more than one-third of serum samples from patients with autoimmune thyroid diseases (ATG), including chronic lymphocytic thyroiditis (CLT) and diffuse toxic goiter (DTG), can *in vitro* cause death of normal thyrocytes isolated from paranodular tissue of individuals with euthyroid nodular goiter [2,3]. Further observations showed that complement-depleted sera from these patients retain this activity. Taking into account the key role of apoptosis in thyrocyte death during ATG and expression of apoptosis receptors on these cells [1,5,6] it can be hypothesized that the antithyroid effect of complement-depleted sera is related to the presence of apoptogenic factors, in particular, soluble FAS ligand (sFASL) [5].

Here we studied the cytotoxic effect of serum samples from patients with ATG depending on the content of sFASL and soluble apoptosis receptor (sFAS).

MATERIALS AND METHODS

Serum samples from 92 patients with DTG and 74 patients with CLT were *in vitro* assayed for antithyroid cytotoxicity. The diagnosis was made by clinical criteria, concentrations of thyrotropin and thyroid hormones in the serum, ultrasound examination of the thyroid gland and punction biopsy (some patients). The age of patients was 17-65 years. Each group included an equal number of men and women in various periods of drug therapy. Serum samples from healthy donors served as the control. Normal thyrocytes were isolated from paranodular tissue of the thyroid gland of patients with euthyroid nodular goiter during surgery and cultured as described elsewhere [3].

Serum samples were heated at 56°C for 30 min for complement inactivation. The cells (100,000) were placed in a 96-well flat-bottom plate. The supernatant was removed after 24 h. The test serum (30 μl) and serum-free growth medium (60 μl) were added. The cells were cultured for 48 h. The supernatant with dead and destructed cells was removed. The cells were washed with medium 199 and dried. Living cells adherent to the bottom of the well were fixed with ethyl alcohol for 20 min, stained with 0.1% azure for 30 min, washed with water, and dried. Ethyl alcohol (100 μl per well) was added for homogenous staining.

The number of thyrocytes resistant to the cytotoxic effect of serum samples was estimated by staining with azure. Optical density was measured on an

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Efos multichannel spectrophotometer at 594 nm. The serum was considered to be active, when optical density exceeded the limits of 2.5 standard deviations from the mean extinction of cells incubated with control samples.

Due to technical reasons, sFASL concentration was measured in serum samples from patients with DTG and CLT from another group. It should be emphasized that these groups of examined patients were matched for age and sex.

sFASL and sFAS concentrations in the serum from 24 patients with DTG, 27 patients with CLT, and 5 healthy donors were measured by enzyme immunoassay with sFas Ligand ELISA (BMS 260/2) and sAPO-1/Fas ELISA (BMS 245) reagents, respectively (Bender MedSystems).

RESULTS

The study with the primary culture of normal thyrocytes revealed cytotoxic activity of complement-depleted sera from 33 patients with DTG (36%) and 28 patients with CLT (38%). Since DTG and CLT have different morphological manifestations (thyrocyte proliferation and cell destruction, respectively), it is surprising that the percent of sera with antithyroid activity was similar in groups of patients with these diseases. These data confirm the results of our previous studies on antibody-dependent complement-mediated cytotoxicity of these sera [1-4]. Our results demonstrate the existence of cellular mechanisms counteracting the effect of potentially cytotoxic sera from patients with DTG [2,3].

It is clear that serum samples from patients with ATG contain complement-independent factors capable of causing thyrocyte death *in vitro*. The role of increased sFASL content (apoptogenic factor) in the cytotoxic effect of serum samples from patients with ATG should be evaluated.

The mean content of sFASL in serum samples was similar in patients of different groups (Table 1). At first glance these results contradict our hypothesis. However, cytotoxic activity was detected not in all serum samples from patients with ATG. Moreover, the scatter of the data on serum sFASL concentration in patients with ATG far surpassed that in healthy donors. We estimated the number of serum samples from patients in which sFASL concentration exceeded the mean level in healthy donors more than by 3 standard deviations (Fig. 1).

sFASL concentration in 9 of 24 serum samples from patients with DTG (37%) exceeded the mean level in healthy donors by one order of magnitude (0.641±0.25 ng/ml). sFASL concentration increased to 0.540±0.22 ng/ml in 8 of 27 serum samples from patients with CLT (30%). However, no intergroup differences were revealed in the increased level of serum sFASL and number of patients with high sFASL concentration. It should be emphasized that the incidence of cytotoxicity corresponded to the number of serum samples with high sFASL concentration. Therefore, the same percentage of serum samples from patients with ATG produced the cytotoxic effect on thyrocytes and had increased concentration of sFASL. It is not an accidental coincidence of these indexes in various serum samples. Most likely, the interaction between FAS and FASL plays a role in antithyroid cytotoxicity of complement-depleted sera from patients with ATG. These results are consistent with our hypothesis.

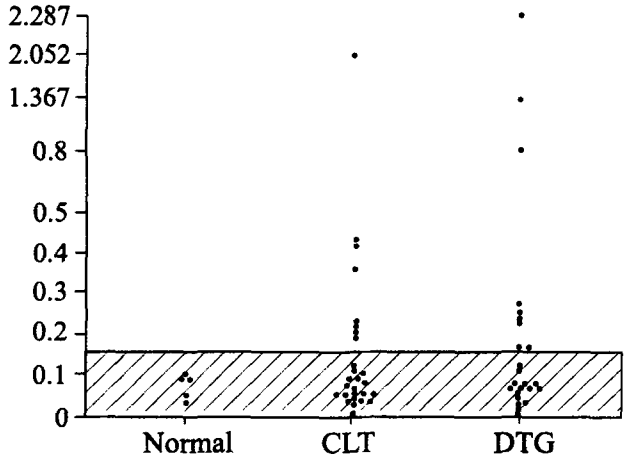

Fig. 1. Fas ligand concentration in the serum from healthy donors (normal) and patients with autoimmune diseases of the thyroid gland. CLT, chronic lymphocytic thyroiditis; DTG, diffuse toxic goiter. Shaded area: $M \pm 3\delta$ (compared to healthy donors).

Previous studies showed that sFASL concentration increases in serum samples from untreated patients with Graves' disease [8]. Our observations showed that sFASL concentration increases only in some serum samples. This is probably related to heterogeneity of groups that included patients with different degree of thyrotoxicosis compensation.

DTG cells were resistant to the cytotoxic effect of serum factors. These cells probably have reduced number of surface antigens (receptors) that mediate the cytotoxic effect [2,3]. APO I (FAS) mediating the apoptogenic action of FASL is probably one of these receptors.

TABLE 1. Concentrations of sFASL and sFAS in Serum Samples from Healthy Donors and Patients with ATG ($M \pm m$)

Group	sFASL, ng/ml	sFAS, pg/ml
Healthy donors (n=5)	0.071±0.012	219.2±33.4
DTG (n=24)	0.282±0.106	267.5±59.0
CLT (n=27)	0.196±0.075	188.0±12.5

Published data show that sFAS concentration is high in serum samples from untreated patients with DTG [7], which probably contributes to protection of cells from the apoptogenic effect of sFASL. However, the resistance of DTG cells cannot be explained by increased sFAS concentration in the serum of these patients. Under these conditions normal thyrocytes would be protected. This hypothesis contradicts the results of our study. Moreover, we revealed no increase in serum sFAS concentration in patients with DTG (Table 1).

Serum sFAS concentration in patients with DTG and CLT did not exceed the limits of 3 standard deviations from the mean level observed in healthy donors. The decrease in the number of receptors on DTG cells mediating the cytotoxic effect of serum samples from patients with ATG is probably related to reduced expression, but not to their "exfoliation" into the blood.

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