The Antibody Response to Endoplasmic Reticulum Stress in Hashimoto’s Thyroiditis

Abstract

Purpose: We aimed to investigate the presence of antibodies (Anti-BIP) against binding immunoglobulin protein (BIP), an endoplasmic reticulum (ER) chaperone with immune modulator and anti-apoptotic effects in Hashimoto’s thyroiditis (HT) patients.

Material and Method: We included sixty-two autoimmune thyroiditis patients, 20 with euthyroid autoimmune thyroiditis, 27 with subclinical hypothyroidism, and 15 with hypothyroid, and a control group of 37 healthy subjects.

Results: No statistically significant difference was determined in anti-BIP levels among the HT subgroups or in comparison with the control group (p=0.889).

Discussion: Although BIP activation has been shown in vitro in thyroid cells, no difference was determined in our study in anti-BIP levels between the HT patient subgroups and the control group. This suggests that antibodies developing against BIP through apoptosis and/or T cell response are either not related to HT or at levels that cannot be determined by measuring serum.

Key words: Hashimoto’s thyroiditis, Anti-BIP, BIP, apoptosis, endoplasmic reticulum stress

Özet

Amaç: Hashimoto tiroiditli hastalarda apoptozis patogenezinde, immün modülatör ve anti-apoptotik etkilere sahip bir endoplazmik retikulum (ER) şaperonu olan immünglobulin bağlayıcı proteine (BIP) karşı oluşan antikorların (Anti-BİP) varlığını araştırmak.

Gereç ve Yöntem: Çalışmaya 20 ötiroid otoimmün tiroidit, 27 subklinik hipotiroidi, 15 hipotiroid hashimoto tiroiditli olmak üzere 62 otoimmün tiroiditli hasta ve 37 sağlıklı kontrol grubu alınmıştır.

Bulgular: Hashimoto tiroiditli (HT) hastaların alt gruplarıyla kontrol grubu arasında ve HT’i alt gruplarının kendi aralarında Anti-BIP düzeyleri birbirleriyle karşılaştırıldığında herhangi bir istatistiksel fark saptanamadı (p=0.889).

Tartışma: Tiroid hücrelerinde in vitro ortamda BIP aktivasyonu gösterilmesine rağmen bizim çalışmamızda HT’li hasta alt gruplarında Anti-BIP düzeyi kontrol grubundan farklı bulunmamıştır. Bu durum HT’de apoptozis ve/veya T hücre cevabında BIP’e karşı oluşan antikorların ilişkisiz olduğu veya serumda tespit edilememekte veya düzeylerde olduğu düşünüldü. Turk Jem 2013; 17: 53-6

Anahtar kelimeler: Hashimoto tiroidit, Anti-BIP, BIP, apoptozis, endoplazmik retikulum stresi
Introduction

Chronic thyroiditis is the most common cause of hypothyroidism (1). It is characterized by diffuse lymphocytic infiltration of the thyroid gland, follicular cell destruction, and eventually hypothyroidism (2). The triggers of autoimmune destruction in Hashimoto’s thyroiditis (HT) are completely unknown. Thyroid cell destruction is gradual, and cell death takes years. The apoptotic process in this disorder has been identified since 1995 (3,4). The main aim of apoptosis is the maintenance of stability among the cell population. In the normal thyroid gland, apoptosis is at very low levels, enough to control the cell cycle and cell destruction (3).

Various physiological and pathological conditions, such as hypoglycemia, pathogenic infection, chemical injury, genetic mutation, increased protein synthesis, and secretion and the flow of calcium into the endoplasmic reticulum (ER), trigger ER stress (5). If stress is prolonged, the injury becomes very severe, and the apoptotic pathway is activated through stimulation of the caspase chain in the ER (6). Binding immunoglobulin protein (BIP), a “heat shock protein 70 class”, is one of the molecular chaperones of the ER. Under conditions that increase ER stress, BIP directly and indirectly participates in apoptosis processes and can play a role as an apoptosis regulator by controlling the cellular response to ER stress-induced cell death (7). BIP protects the cell from apoptosis by blocking caspase activation.

In addition to chaperone activities, BIP also has immunological functions, such as in cytotoxic T cell responses and in non-classical antigen presentation. With excessive BIP expression, BIP becomes partially expressed on the cell surface. It is targeted by specific regulator T cells and establishes the stimulation of suppressor T cells. This immune response permits the suppression of immune hyper-reactivity and response (8).

In thyroid cells, thyrotropin (TSH) stimulation increases the ER chaperone BIP by enhancing thyroglobulin (Tg) synthesis. Under physiological conditions, BIP plays an important role in the early period of Tg polypeptide folding. By binding to Tg aggregates and unfolded Tg monomers, it makes Tg ready to form the three-dimensional protein needed for transport from the ER (9).

Anti-BIP antibodies reduce BIP levels and in turn BIP’s protective effects against inflammatory events and the blocking of effects against apoptosis (10). Antibodies to BIP, with its immune modulator and anti-apoptotic properties, may contribute to the progression of apoptotic mechanisms in HT. The aim of our study was to investigate the presence of anti-BIP antibodies in the serum of HT patients. The presence of these antibodies may shed light on the apoptosis mechanisms in HT and the development of new treatments associated with the apoptotic pathway in the disease.

Materials and Methods

Patients

Ninety-nine patients were enrolled in the study. These were selected from among patients attending the endocrinology outpatient clinic at Başkent University Hospital, Turkey, between 2006 and 2007. A control group (Group 1) of 37 healthy volunteers was established. The patient group consisted of 62 patients with autoimmune thyroiditis, 20 with euthyroid autoimmune thyroiditis (Group 2), 27 with subclinical hypothyroidism (Group 3) and 15 with overt hypothyroidism HT (Group 4). The study was conducted in accordance with the Ethical Guidelines of the Baskent University Faculty of Medicine and was approved by the Institutional Review Board.

Diagnosis of HT patients was made on the basis of serum levels of anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-Tg), TSH, free-T4 (FT4), free-T3 (FT3), thyroid ultrasound, and clinical findings. No infectious or autoimmune disease was present in any patient. None of the patients enrolled had any history of any drug use. Among patients with hypothyroidism, TSH was high, FT4 was low and FT3 was low or within normal limits. In the subclinical hypothyroid patients, TSH was high and FT4-FT3 levels were normal. In the euthyroid patients, TSH, FT4 and FT3 were all within normal levels. One or both of anti-TPO and anti-Tg were high in all three groups. Thyroid ultrasound exhibited a hypoechoic pattern in 75% of patients with autoimmune thyroiditis.

None of the subjects in the control group had any history of infectious or autoimmune disease or drug use. TSH, FT3, FT4, anti-TPO and anti-Tg levels and ultrasound images were all normal in all participants.

Methods

Blood samples were taken after 12-h fasting. After coagulation, the specimens were centrifuged at 3000 rpm for 15 min. Sera were separated and hormone levels and anti-Tg were investigated at once, while sera were stored for anti-TPO and anti-BIP at −70 °C for mass assay. For evaluating thyroid function, FT4, TSH and anti-Tg were measured using immunochromeluminescent assays on an automated analyzer (Bio-DPC, USA). For TSH assay, a third generation kit was used, analytic sensitivity being regarded as 0.004 μIU/ml and reference interval as 0.4-4 μIU/ml (intra-assay CV: 3.8%, interassay CV: 4.6%). For FT4, the reference intervals are 0.8-1.9 ng/dl. The reference interval for anti-Tg is 0-40 IU/ml. Anti-TPO was investigated using the indirect immunofluorescence method (Euroimmun, Germany). Analysis was performed with 1/100, 1/320 and 1/1000 titrations. Titrations below 1/100 were regarded as negative and values above that as positive.

Anti-BIP Enzyme-Linked Immunosorbent Assay

Each well in the enzyme-linked immuno-sorbent assay plate was covered with a buffer so as to contain pH 8.2. 10 μg/ml BIP, 10% goat serum was added to block unwanted nonspecific bindings. The plate was covered and kept at room temperature for 24 h, and 1/100 diluted patient sera were added and kept overnight at +4°C. To each well was added 1% bovine serum and 0.05% Tween-20, and conjugate was diluted in 1/20,000 phosphate buffered saline buffer. In the final stage, color formation was observed with the addition of 3.3’5.5’ tetramethyl benzidine. The plaque was read at 450 nm and patient and control specimen absorbances were recorded.

Statistical Analysis

SPSS 20.0 was used for statistical analysis. Analysis of variance was used to test the differences between groups for normally distributed data. The Tukey test was used post hoc to test was performed to
Results

The control group consisted of 37 healthy volunteers. Sixty-two HT patients, 20 with euthyroid autoimmune thyroiditis, 27 with subclinical hypothyroidism and 15 with overt hypothyroidism constituted the patient groups. There was no difference between the four groups in terms of age and sex. Patient characteristics are given in Table 1.

No difference was determined between groups 2, 3 and 4 or compared with Group 1 in terms of anti-BIP levels (p=0.889). A comparison of the groups' anti-BIP levels is given in Table 1 and Figure 1.

Anti-BIP was not found to correlate with anti-TPO, anti-Tg, TSH, and FT4 (p>0.05).

Discussion

Hashimoto’s thyroiditis is characterized by a lymphocyte infiltrate, the presence of thyroid auto-antibodies, and destruction of the thyroid gland. The reasons for the initiation of the destruction of the thyroid gland are completely unexplained. In recent years, the relationship between apoptotic processes and the pathogenesis of HT have attracted attention (3,4). Physiological stress conditions do not lead to apoptosis. However, when stress is prolonged, and homeostasis cannot be established, mutant proteins in the ER accumulate and the apoptotic pathway containing the caspase cascade in the ER is activated, leading to cell death (6). Under stress conditions, BIP expression prevents the aggregation of unfolded proteins by binding to them and promoting folding (11).

Figure 1. Comparison of Anti-BIP levels between the groups (p=0.889)

Table 1. General patient characteristics and comparison of Anti-BIP levels between the groups

|                      | Group 1 | Group 2 | Group 3 | Group 4 | p    |
|----------------------|---------|---------|---------|---------|------|
| Gender (F/M)         | 31/6    | 19/1    | 23/4    | 14/1    | 0.544|
| Age (years)          | 32.27±8.47 | 34.90±11.86 | 38.56±11.18 | 38.93±10.46 | 0.146|
| TSH (µIU/mL)         | 1.43±0.89  | 11.96±0.90 | 13.12±13.95 | 47.19±33.92 | <0.001|
| FT4 (ng/dL)          | 1.325±0.22 | 1.20±0.16 | 1.04±0.21 | 0.51±0.09 | <0.001|
| Anti-TPO (+)         |          |         |         |         | <0.001|
| <1/100               | 100%/137 | 20%/4   | 7.4%/12 | 13.3%/12 |        |
| 1/100                | 0%       | 5%/1    | 3.7%/11 | 0%/10   |        |
| 1/320                | 0%       | 10%/2   | 14.8%/14 | 6.7%/11 |        |
| 1/1000               | 0%       | 65%/13  | 74.1%/20 | 80%/12  |        |
| Anti-Tg (IU/mL)      | 27.59±12.57 | 288.71±350.2 | 345.8±364.41 | 549.3±427.5 | <0.001|
| Anti-BIP (µg/mL)     | 0.28±0.09 | 0.28±0.09 | 0.30±0.84 | 0.29±0.12 | 0.889|

Group 1: control group, group 2: euthyroid autoimmune thyroiditis, group 3: subclinical hypothyroidism, group 4: overt hypothyroidism, F: female, M: male, TSH: Thyrotropin, FT4: Free-T4, FT3: free-T3, Anti-TPO: Anti-thyroid peroxidase, Anti-Tg: Anti-thyroglobulin, Anti-BIP: Antibodies against binding immunoglobulin protein.
Our theory was that anti-BIP antibodies may play a role in apoptotic events in the autoimmune process in HT. However, we found no difference in terms of anti-BIP antibody levels between controls and those involving euthyroid, subclinical and overt hypothyroid phases. This shows that anti-BIP antibodies are unrelated to apoptosis-related events in HT, at least at the serum level. In vitro studies of thyroid tissue suggest that ER stress increases in the autoimmune process [12]. One reason for a lack of these antibodies in the serum may be that an increase in anti-BIP antibodies at the tissue level was not sufficient to be measured in the serum and may depend on a local activation process.

The reason why we divided HT patients into euthyroid, subclinical and overt hypothyroidism subgroups was to analyze the different phases related to disease activity. However, we found no difference either among the subgroups or compared with the control group. Furthermore, we did not find a correlation between anti-BIP and levels of thyroid auto-antibodies.

Thyroglobulin synthesis is known to increase with the stimulation of TSH in thyroid cells. Under physiological conditions, BIP plays an important role in the early period of Tg polypeptide folding [9]. In our study, although TSH was high in our overt hypothyroid and subclinical hypothyroid patients, the anti-BIP antibody level was not different from that of the control group.

BIP enables the stimulation of suppressor T cells. This immune response permits the suppression of immune hyperreactivity and response [8]. In CTLA-4-related mutations in HT, the suppressor T cell response is impaired. The fact that we were unable to show anti-BIP antibodies in our study suggests that suppressor T cell dysfunction in HT is unrelated to anti-BIP antibodies. We hypothesized that antibodies might develop to BIP, shown to be expressed in thyroid tissue, in the same way that antibodies develop to Tg, thyroid peroxidase, Na/I transporter and TSH receptor in HT. However, we were unable to show a difference in anti-BIP in our HT patient sera compared to those of the control group.

BIP possesses cellular protection properties with suppressive characteristics in cytotoxicity-mediated cell destruction and in ER stress-related apoptotic pathways. Our hypothesis was that antibodies developing against BIP could eliminate this protective effect in HT. Although BIP activation has been shown in thyroid cells, no difference was found in our study in anti-BIP levels between our HT subgroups and the control group. This suggests that antibodies developing against BIP are not related to apoptosis and/or T cell response in HT, or that they are at levels in serum that cannot be determined.

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