Expression and distribution of S-100 protein, CD83 and apoptosis-related proteins (Fas, FasL and Bcl-2) in thyroid tissues of autoimmune thyroid diseases

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The pathogenesis of autoimmune thyroiditis has been studied in detail in an animal model (autoimmune prone BB DP rats) that spontaneously develops this condition (Simons et al. 2000). That study showed that the pathogenesis of autoimmune failure of the thyroid is a multistep process requiring several genetic and environmental abnormalities (or variants) to converge before full blown disease develops.

In the early phases of the disease, two immune processes can be distinguished. Firstly, the afferent phase, characterized by an intrathyroid accumulation of dendritic cells (DCs). Blood monocytes are an important precursor population of these cells and are able to differentiate into various subsets of DCs (Peters et al. 1996). DCs are antigen-presenting cells (APC) par excellence and are essential for the stimulation of naïve T cells which leads to the sensitization and clonal expansion of the latter (Drexhage et al. 1999). Increased numbers of the major histocompatibility complex (MHC) class II-positive DCs have been found both inside and outside lymphocytic accumulations in the thyroid of GD or HT patients (Kabel et al. 1998) and in the thyroid of the animal models (Naqayama 2005; Marinkovic et al. 2006).

Consequently, a central phase in which the lymphocytes react to the presented autoantigens is characterized by an apparently uncontrolled production of autoreactive CD4+ T cells, CD8+ cytotoxic T cells and autoantibodies of the immunoglobulin G (IgG) class. Initially, this production of autoreactive cells and autoantibodies takes place in the draining lymph nodes. Later, however, lymphoid tissue often develops locally in the thyroid gland, at least in the BB-DP rat (Banchereau et al. 1998). This local lymphoid tissue has a high degree of histologic architecture with clearly distinguishable T-cell areas, B-cell follicles with germinal centers, and areas and cords of plasma cells in the periphery of the lymphoid tissue radiating between the thyroid...
folicles. The plasma cells produce antithyroglobulin (anti-Tg) antibodies.

The Fas/FasL system is one of an expanding family of receptor-ligand pairs involved in cell fate determination in a variety of cells (Nagata et al. 1995). When FasL binds to Fas on Fas-sensitive target cells, the target cells die by apoptosis (Nagata et al. 1995). FasL expression in non-lymphoid tissue is important for protecting immune-privileged sites from immune-mediated damage (Griffith et al. 1995; Zhang et al. 2005). On the other hand, the Bcl-2 protooncogene is the prototype of a family of genes that inhibit apoptotic cell death induced by various stimuli, such as growth factor deprivation (Cory et al. 1995).

There is considerable evidence that Fas/FasL-mediated apoptosis plays an important role in the active stage of the autoimmune process in both HT and GD (Mysliwiec et al. 2006, 2007; Mitsiades et al. 1998; Hiromatsu et al. 1999). The expression of Bcl-2 may render thyrocytes resistant to Fas/FasL-mediated elimination and may thus be involved in the pathogenesis of HT and GD (Hiromatsu et al. 1999).

It is known that S-100 protein is a non-specific marker of DCs and that CD83 antigen is a specific marker of activated and mature human DCs; both have been used to identify DCs. The S-100 marker has been examined extensively in ATD, but little is known about the expression and distribution of CD83 antigen in the thyroid tissues of ATD. Does the number of maturation stages of DCs increase in ATD? This study used immunohistochemical methods to follow the expression and distribution of S-100 protein, CD83 and apoptosis-related proteins (Fas, FasL and Bcl-2) in the thyroid tissues of different ATD and to determine the role of DCs and apoptosis in the pathogenesis of ATD and to find possible connections between them.

Materials and Methods

Subjects and thyroid tissues

30 HT patients (females, aged 31-63 years) and 30 GD patients (females, aged 28-65 years) were involved in this study. Normal thyroid tissues adjacent to thyroid follicular adenoma (TFA), obtained from 30 subjects with TFA (females, 26-60 years), were assigned as the control. The patients were admitted to the First Hospital of Shantou University Medical School between 2001 and 2006. Diagnosis was made from physical examination of the patients and laboratory testing and confirmed by histological examination (hematoxylin and eosin staining) of the thyroid tissue samples. Informed consent was given by all of the patients and control subjects after explaining the nature and purpose of the study. The thyroid tissue specimens were collected during surgical operation. All samples were fixed in 10% buffered formalin, embedded in paraffin, and cut into 4 µm sections.

Detection of antibodies against thyroid peroxidase (TPO-Ab), thyroglobin (Tg-Ab) and the thyroid hormone receptor (TR-Ab)

Blood samples were collected from each patient. Serum TPO-Ab, Tg-Ab and TR-Ab were determined by RIA according to the instructions supplied with the radioimmunoassay kit (Beijing North Institute of Biological Technology, Beijing, China).

Immunohistochemical staining

Immunohistochemical staining was used to detect S-100 protein, Fas, FasL and Bcl2. Tissue sections were firstly incubated in phosphate-buffered saline (PBS) containing the primary antibody (rabbit anti-human polyclonal antibody to S-100 protein (PharMingen International, CA); Fas (Sc-714-G, Santa Cruz); FasL (Sc-534-G, Santa Cruz); or mouse anti-human monoclonal antibody to Bcl-2 (Sc-509, Santa Cruz) at 4°C overnight, followed by incubation with biotin-labeled secondary antibody for 1h and then with ABC complex for 30 min. The sections were developed in DAB and then mounted and observed under a microscope. A known sample from a patient with breast cancer was used as a positive control, and PBS was used instead of the primary antibody as the negative control.

The staining of CD83 was performed as described by Chen et al. (2000) with some modifications. Formalin-fixed, paraffin-embedded thyroid specimens were washed three times in PBS and treated with pepsin (0.5% in 0.01N HCl) for 20 min at 37°C before staining for CD83. The specimens were then treated with normal goat serum for 20 min to block non-specific binding. Mouse anti-human monoclonal antibodies as primary antibody (PharMingen), diluted 1:100, were then added and incubated overnight. The sections were washed 3x with PBS and reincubated with biotinylated goat anti-mouse immunoglobulin (1:200, DAKO, Denmark) at room temperature for 1 h. After a wash in PBS, sections were soaked in alkaline phos-
phatase-conjugated streptavidin (DAKO) and washed. New fuchshin (DAKO) was then used as chromogen. Hematoxylin was used as a counter stain. A known sample from a patient with hepatocellular carcinoma was used as a positive control. Negative control slides were processed with PBS instead of the primary antibody, but included all other steps of the procedure.

**Positive staining estimation**

Expression of Fas, FasL and Bcl-2 protein was seen on the membrane and/or in the cytoplasm of thyroid follicular cells and/or infiltrating lymphocytes. Expression of S-100 protein was noticed in the nucleus and/or in the cytoplasm of DCs, appearing as brown granules, and the expression levels were stronger than background staining. Expression of CD83 showing as red granules was observed on the membrane and/or in the cytoplasm of DCs. CD83-positive cells were distributed throughout the specimens. The intensity of positive staining of S-100, Fas, FasL and Bcl-2 protein was evaluated subjectively by 2 independent observers using a 10x lens in 10 randomly selected fields of each specimen. Each observer made an average of the percentages of the 10 fields and then the mean of the two scores was made. Positive staining of Fas, FasL and Bcl-2 protein in the thyrocytes is expressed as the percentage of positive thyrocytes to the total number of thyrocytes in each field. Positive staining of Fas, FasL and Bcl-2 protein in the lymphocytes is expressed as the percentage of positive lymphocytes to the total number of TILs in each field.

S-100 protein-positive cells are shown as the ratio of a total of 100 infiltrating cells. The percentages of CD83+ cells are shown as the total number of positive cells/specimen.

**Flow cytometry**

DCs were enriched from blood as described previously (Chen et al. 2000; Ninomiya et al. 1999). The percentage of CD83-positive DCs in the DC population was estimated by flow cytometry. The DCs were suspended in a solution of PBS, 1% fetal calf serum, and 0.1% sodium azide with saturating amounts of fluorescein isothiocyanate-conjugated mouse monoclonal antibody to human CD83 (HB15e; BD PharMingen) or isotype matched controls for 30 min at 4°C. After two washes in fresh buffer, the percentage of CD83-positive DCs was estimated on a FACStarp1 (Beckton Dickinson) after removing the dead cells and contaminating lymphocytes.

**Statistical analysis**

The data were expressed as mean±SD. The statistical analysis was done by unpaired or paired t-tests. Positivities for S-100, Fas, FasL and Bcl-2 protein, CD83+ in TFA, and HT and GD are shown as percentages, and comparisons were made by the x2 test. P-values less than 0.05 are considered to indicate statistical significance. The SPSS 10.0 statistical program was used for the calculations.

**Results**

**Levels of TPO-Ab, Tg-Ab and TR-Ab**

Serum TPO-Ab (67.3±11.6%) and Tg-Ab (59.8±10.1%) in HT were, respectively, higher than in GD (28.4±5.7%, 23.1±4.9%) or in TFA (6.1±3.4%, 7.2±4.6%)(p<0.01). Serum TR-Ab in GD (16.3±5.6U/L) was higher than in HT (4.8±2.3U/L) or in TFA (2.5±1.2U/L)(p<0.01). The data are shown in Table 1.

**Expression of S-100**

The expression of S-100 protein was detected in the nucleus and cytoplasm. TFA tissue seldom expresses S-100 protein (with staining intensity 0.95±0.64) (Figure 1A). Compared with TFA, the expression of S-100 protein in HT and GD was elevated (with staining intensity 4.2±3.1 and 3.9±2.8, respectively) (Figure 1B,C and Table 2)(p<0.001)

| Table 1. Levels of TPO-Ab, Tg-Ab and TR-Ab in the TFA, HT and GD groups. |
|-----------------------------|-----------------|-----------------|-----------------|
| **Group** | n | TPO-Ab (%) | Tg-Ab (%) | TR-Ab (U/L) |
|-----------------------------|-----------------|-----------------|-----------------|
| TFA | 30 | 6.1±3.4 | 7.2±4.6 | 2.5±1.2 |
| HT | 30 | 67.3±11.6* | 59.8±10.1* | 4.8±2.3 |
| GD | 30 | 28.4±5.7 | 23.1±4.9 | 16.3±5.6** |

*, p<0.01 vs GD or TFA; **, p<0.01 vs HT or TFA.

| Table 2. S-100 expression in the TFA, HT and GD groups. |
|-----------------------------|-----------------|-----------------|
| **Group** | n | Total prevalence (%) | Staining intensity (x±SD) (%) |
|-----------------------------|-----------------|-----------------|
| TFA | 30 | 100 | 0.95±0.64 |
| HT | 30 | 100 | 4.2±3.1* |
| GD | 30 | 100 | 3.9±2.8** |

*, vs TFA, p<0.001; **, vs TFA, p<0.001.
**Expression of CD83**

The CD83-positive DCs were distributed in infiltrating lymphocytes. CD83 was expressed in the cytoplasm. CD83 was seldom expressed in TFA tissues (with positive rate of 30%, percentage 5.19±8.08%) (Figure 1D), but showed greater expression in HT (with positive rate of 75%, percentage 22.58±13.96%) (Figure 1E) and GD tissues (with positive rate of 30%, percentage 5.19±8.08%) (Figure 1D), but showed greater expression in HT (with positive rate of 75%, percentage 22.58±13.96%) (Figure 1E) and GD tissues (with positive rate of 30%, percentage 5.19±8.08%) (Figure 1D), but showed greater expression in HT (with positive rate of 75%, percentage 22.58±13.96%) (Figure 1E) and GD tissues (with positive rate of 30%, percentage 5.19±8.08%) (Figure 1D), but showed greater expression in HT (with positive rate of 75%, percentage 22.58±13.96%) (Figure 1E) and GD tissues (with positive rate of 30%, percentage 5.19±8.08%) (Figure 1D), but showed greater expression in HT (with positive rate of 75%, percentage 22.58±13.96%) (Figure 1E) and GD tissues (with positive rate of 30%, percentage 5.19±8.08%) (Figure 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Figure 1. Expression of S-100 protein and CD83 in thyroid follicular adenoma, Hashimoto's thyroiditis and Graves' disease. Expression of S-100 protein was detected by immunohistochemical staining in TFA (A, ×400), HT (B, ×400) and GD (C, ×400) and those of CD83 in TFA (D, ×400), HT (E, ×400) and GD (F, ×400).
(with positive rate of 80%, percentage 29.92±14.43%) (Figure 1F), percentages which were significantly higher than that in TFA \( (p<0.05, \) Table 3).

**Expression of Fas, FasL and Bcl-2**

All samples expressed Fas, mainly on the cell surface and cytoplasm (Figure 2A-C). TFA thyrocytes expressed moderate Fas (Figure 2C) and minimal or absent FasL (Figure 3C). GD thyrocytes expressed less Fas (Figure 2B) and FasL (Figure 3C) than did HT thyrocytes (Figures 2A, 3A) \( (p<0.05, \) whereas GD TILs showed higher levels of Fas/FasL (Figures 2B, 3C) than did HT TILs (Figures 2A, 3B) \( (p<0.05). \) GD thyrocytes expressed increased levels of Bcl-2 (Figure 4B) \( (p<0.05) \) compared to the low levels detected in HT thyrocytes (Figure 4A). The opposite pattern was observed in GD (low Bcl-2) (Figure 4B) and HT (high Bcl-2) (Figure 4A) TILs.

The results mentioned above are summarized in Tables 4 and 5.

**Table 3. CD83 expression in the TFA, HT and GD groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Total prevalence (%)</th>
<th>Staining intensity (x±SD) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFA</td>
<td>30</td>
<td>9/30 (30)</td>
<td>5.19±0.08</td>
</tr>
<tr>
<td>HT</td>
<td>30</td>
<td>23/30 (77)</td>
<td>22.58±13.96**</td>
</tr>
<tr>
<td>GD</td>
<td>30</td>
<td>24/30 (80)</td>
<td>29.92±14.43**</td>
</tr>
</tbody>
</table>

HT vs TFA \( *p<0.05, **p<0.001<GD \)

**Table 4. Prevalence of positive Fas, FasL and Bcl-2 in the HT, GD and TFA groups.**

<table>
<thead>
<tr>
<th></th>
<th>HT Thyroid Follicles</th>
<th>HT Lymphocytes</th>
<th>GD Thyroid Follicles</th>
<th>GD Lymphocytes</th>
<th>TFA Thyroid Follicles</th>
<th>TFA Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fas</td>
<td>100% (30/30)</td>
<td>100% (30/30)</td>
<td>100% (30/30)</td>
<td>100% (30/30)</td>
<td>100% (30/30)</td>
<td>100% (30/30)</td>
</tr>
<tr>
<td>FasL</td>
<td>100% (30/30)</td>
<td>100% (30/30)</td>
<td>100% (30/30)</td>
<td>100% (30/30)</td>
<td>83% (25/30)*</td>
<td>83% (25/30)</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>73% (22/30)</td>
<td>63% (19/30)</td>
<td>97% (29/30)**</td>
<td>97% (29/30)</td>
<td>83% (25/30)</td>
<td>83% (25/30)</td>
</tr>
</tbody>
</table>

* TFA vs HT and GD respectively, \( p<0.05; **GD vs HT, p<0.01.*

**Table 5. Staining intensity of Fas, FasL and Bcl-2 in HT, GD and TFA (n=30) groups.**

<table>
<thead>
<tr>
<th></th>
<th>HT Thyroid Follicles</th>
<th>HT Lymphocytes</th>
<th>GD Thyroid Follicles</th>
<th>GD Lymphocytes</th>
<th>TFA Thyroid Follicles</th>
<th>TFA Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fas</td>
<td>76.31±15.79**</td>
<td>43.42±22.85**</td>
<td>54.26±22.53</td>
<td>68.42±22.85</td>
<td>35.75±12.89</td>
<td>35.75±12.89</td>
</tr>
<tr>
<td>FasL</td>
<td>60.25±26.51*</td>
<td>46.10±25.80*</td>
<td>50.10±28.36</td>
<td>60.10±26.10</td>
<td>26.08±20.73</td>
<td>26.08±20.73</td>
</tr>
</tbody>
</table>

* vs GD, \( p<0.05; ** vs GD, p<0.01. \* vs GD and TFA, \( p<0.05.*
Percentage of CD83-positive DCs in blood from patients with TFA, GD and HT

The percentage of CD83-positive DCs in GD and HT were 3.3±0.8% and 3.1±1.0% (mean±SD, n=30) of the total DCs enriched from blood by using GM-CSF and IL-4 similar to TFA (2.8±0.9%, n=30, p>0.05).

Discussion

Dendritic cells are effective antigen-presenting cells (APCs) that can stimulate both primary and secondary T- and B-cells. DCs exhibit characteristic morphology with multiple extending cytoplasmic processes, express major histocompatibility complex class I and II molecules as well as CD40 and CD80, and are S-100 protein positive (Mule 2000). DCs are well known to provoke organ-specific autoimmune diseases (Drakesmith et al. 2000; Turley et al. 2002; Weir et al. 2002). The detection of DCs in lesions associated with numerous autoimmune diseases (Turley et al. 2002), including thyroiditis (Canning et al. 2003), have strongly argued for DCs involvement in the initiation of autoimmunity. The maturation stage of DCs seems to play a pivotal role in this process: under homeostatic conditions, immature DCs are believed to continually transport autoantigens to draining lymph nodes, process and present them to cognate T cells in a substimulatory context, leading to T-cell tolerance (Turley et al. 2002; Hawiger et al. 2001; Lutz et al. 2002; Veeraswamy et al. 2003). Under the influence of endogenous 'danger signals' released by tissues undergoing stress, damage or abnormal death, or exogenous danger signals elaborated by pathogens, DC undergo maturation (Gallucci et al. 2001). After uptake of the antigen, primed DCs migrate to lymphoid organs and enhance antigen-targeted presentation to the immune system. DCs

Figure 3. Representative staining pattern of FasL in thyroid follicles (A, x400) and lymphocytes (B, x400) of Hashimoto’s thyroiditis and in Graves’ disease (C, x400). Note that it is negative in thyroid follicular adenoma (D, x100).
play a control role not only in the initiation but also in the maintenance of ATD (Naqayama et al. 2005). It is known that S-100 protein is a non-specific marker of DCs, and CD83 antigen is a specific marker of mature, activated human DCs (Chen et al. 2000). In animal models, the first sign of a developing autoimmune reaction is in fact an increase in the number of DCs in the thyroid, as well as a local homotypic clustering of these cells (Naqayama, 2005). The fate of DCs accumulated in tissues is to enter the lymphatics and then travel to the draining lymph nodes while transporting antigens. Homotypic interactions play a role in activation and further maturation of DCs (Delemarre et al. 2001). What attracts these DCs to the thyroid tissues? First, attraction signals may simply be inflammatory in nature. Early nonspecific necrosis of thyrocytes due to toxins (e.g. iodine) and perhaps viral or bacterial infection, with the concomitant release of proinflammatory factors and self antigens has been described as an eliciting factor. Second, DCs may accumulate not to exert a function of defense nor in removal of cell debris, but to regulate growth and function of neighboring thyrocytes. It is relevant to note that DCs are normal constituents of the thyroid, and that the cells have been proven to regulate the growth and function of thyrocytes in vitro (Simons et al. 1998a) via IL-1 and IL-6 (Simons et al. 1998b). Moreover, DCs are responsive to TSH (because they express the TSH receptor) and to thyroid hormones, and particularly to produce proinflammatory cytokines such as IL-1β and IL-12 under such conditions (Bagriacik et al. 2000).

The data showed that S-100 protein positive DCs and CD83+ DCs accumulate in the thyroid tissues of HT and GD patients. These DCs are in close contact with thyroid follicular cells or infiltrating lymphocytes. This shows that in the thyroid tissue of autoimmune thyroid diseases, the number of DCs is abnormally increased, and these DCs may actively take part in the initiation and maintenance of autoimmune thyroid diseases.

Nowadays, CD83+ DCs are considered as mature and activated dendritic cells which also can express high level of costimulatory factors (such as CD80 and CD86). These CD83-positive DCs possess very powerful antigen presenting ability (Pesce et al. 2002). Studies (Braley-Mullen et al. 1997) have shown that the number of immune-active DCs were increased in the thyroid tissues of HT and GD and were related to the development of the diseases. Zhang Huijang (Zhang et al. 1996) had discovered that the extent of infiltration of DCs in the thyroid tissues of HT was highly positively correlated with

![Figure 4. Representative staining pattern of Bcl-2 in Hashimoto’s thyroiditis (A, x400), in Graves’ disease (B, x200) and in thyroid follicular adenoma (C, x200).]
the value of Tg-Ab and TPO-Ab. This suggests that the autoimmune failure of the thyroid and the high level of auto-antibodies are correlated with the increased number of DCs. This study showed that the number of CD83+ DCs was increased in the thyroid tissues of HT and GD but the thyroid tissues of TFA seldom expressed CD83. To see if the number of CD83-positive DCs is also increased in the peripheral blood, we carried out a flow cytometric analysis to estimate the frequencies of CD83-positive DCs in the peripheral blood. We found almost similar frequencies of CD83-positive DCs in peripheral blood from TFA, GD and HT. The similar frequencies of CD83-positive DCs in TFA, GD and HT implies that the activation and maturation of DCs occur only locally in the thyroid tissue or in the neighbouring lymphoid organs of ATD but not in the peripheral blood, and also implies that the increased number of CD83-positive DCs do not come from the peripheral blood. Although the exact significance of these findings remain to be clarified, an importance of tissue infiltrating DCs in these pathological conditions is now evident. The findings are consistent with others (Braley-Mullen et al. 1997; Quadbeck et al. 2002; Zhang et al. 1996; Mizutori et al. 2006; Li et al. 2006).

HT and GD are both considered to be autoimmune thyroid disorders. In HT, the infiltration of lymphocytes and the destruction of thyrocytes are prominent histological features and hypothyroidism is often the result. In contrast, GD is characterized by the hyperplasia of thyrocytes that results from stimulation with anti-TSH receptor autoantibodies and result in hyperthyroidism. How to explain the above phenomenon? Does apoptosis of thyrocytes play a role in the pathogenesis of autoimmune thyroid diseases?

By formation of a death-inducing signaling complex and initiation of a signaling cascade of caspases, Fas ligand (FasL) induces apoptosis of Fas-expressing cells (Thome et al. 2001; Budd et al. 2006). It also plays an important role in many human and murine autoimmune diseases, such as Hashimoto’s thyroiditis and Graves’ disease (Stassi et al. 2002.), multiple sclerosis (Semra et al. 2001), experimental allergic encephalomyelitis (EAE) (Suvannavejh et al. 2000), type 1 diabetes (Charles et al. 2006), and G-EAT (Stassi et al. 2002; Wei et al. 2003). The Fas/FasL pathway can function to induce autoimmune damage (Mahiou et al. 2001), and also to shut down autoimmune responses (Wei et al. 2004).

Bcl-2 is another apoptosis-related protein. Recently, Bcl-2 was shown to be down-regulated during the early events that lead to programmed cell death (Suzuki et al. 1996). Bcl-2 may be an inhibiting molecule and play an important role in the balance between apoptosis promotion and inhibition.

We studied the role of the apoptosis-related molecules Fas, FasL and Bcl-2 in ATD, such as Hashimoto’s thyroiditis and Graves’ disease. We found that follicular cells from normal thyroid tissues adjacent to follicular adenoma (TFA) expressed moderate Fas but minimal FasL. These have been corroborated by other studies (Mitsiades et al. 1998; Arscott et al. 1997). Another study (Giordano et al. 1997) showed the absence of Fas and the presence of FasL in non-autoimmune thyroid tissues. This discrepancy may be explained by the use of different nontoxic goiters as normal thyroid tissues. Follicles adjacent to lymphocytes in HT exhibited stronger positive Fas/FasL staining than those in GD and TFA, but lymphocytes in HT showed weaker positive Fas and FasL staining than those in GD. Bcl-2 immunostaining was similar in GD and normal control (TFA), but follicular cells in the vicinity of lymphocytes of HT tissues exhibited significantly weaker Bcl-2 staining. The opposite pattern was observed in GD (low Bcl-2) and HT (high Bcl-2) TILs. Interestingly, this study found that Fas and FasL expressed on various follicular cells and lymphocytes. On the other hand, Bcd-2 expressed only on the normal or hyperplasic cells but not on those destructed cells or hypoplastic cells.

The concomitant up-regulation of FasL in affected areas of HT tissues suggests that the Fas/FasL system is important in the apoptotic process in this setting and in the pathogenesis of HT.

Our findings suggest that in GD thyroids, the regulation of Fas/FasL/Bcl-2 favors apoptosis of infiltrating lymphocytes. Moreover, the reduced levels of Fas/FasL and increased levels of Bcl-2 should favor thyrocyte survival and hypertrophy associated with stimulatory thyroid-stimulating hormone receptor antibodies. In contrast, the regulation of Fas/FasL/Bcl-2 expression in HT can promote thyrocyte apoptosis via homophylic Fas-FasL interactions, and a gradual reduction in thyrocyte numbers leading to hypothyroidism. Fas-mediated apoptosis may be a general mechanism of cell damage in destructive organ-specific autoimmunity.
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References


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Wei Y, Chen K, Sharp GC, Braley-Mullen H. FLIP and FasL expression by inflammatory cells vs. thyrocytes can be predictive of chronic inflammation or resolution of autoimmune thyroiditis. Clin Immunol 2003; 108:221-33.


