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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Previous studies have shown that dendritic cells (DCs) and apoptosis-related proteins play a critical role in the pathogenesis of autoimmune thyroid diseases (ATD).This study was designed to investigate the expression and distribution of S-100 protein, CD83 and apoptosis-related proteins (Fas, FasL and Bcl-2) in the thyroid tissues of ATD and their role in ATD pathogenesis as determined by immunochemical staing techniques and other methods. Pathological tissues of 30 patients with Hashimoto's thyroiditis (HT), 30 patients with Graves' disease (GD) and 30 cases of thyroid follicular adenoma (TFA, as control) were used for this study. A higher expression of S-100 in HT (4.2±3.1%) and GD (3.9±2.8%) vs TFA (0.95±0.64%) (p<0.001). was observed as well as a higher expression of CD83 in HT (22.58±13.96%) and GD (29.92±14.43%) *vs* TFA (5.19±8.08%) (p<0.001). HT thyrocytes adjacent to thyroid infiltrating lymphocytes (TILs) showed greater increases in the levels of Fas and FasL than did the GD thyrocytes while HT TILs exhibited lower expression of Fas and FasL than did the GD TILs. GD thyrocytes expressed increased levels of the antiapoptotic protein Bcl-2 as compared to the low levels detected in HT thyrocytes. An opposite pattern was observed in the TILs in GD (low expression of Bcl-2) and HT (high expression of Bcl-2). The findings suggest that the high expression of DC markers is related to the pathogenesis of HT and GD. Up-regulation of both the number and matured functions of DCs may lead to the presentation of more antigens to lymphocytes which are related to the development of autoimmune thyroid diseases. The regulation of Fas/FasL/Bcl-2 in GD favors apoptosis of infiltrating lymphocytes and thyrocyte survival. The regulation of Fas/FasL/Bcl-2 in HT may promote thyrocyte apoptosis leading to hypothyroidism.

Key words: Hashimoto's thyroiditis (HT), Graves' disease (GD), S-100, CD83, apoptosis-related proteins, immunohistochemistry.

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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

follicles. 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 conncections 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. Diagnisis 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 peroxidate (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-labled 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 are 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 calclations.

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 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

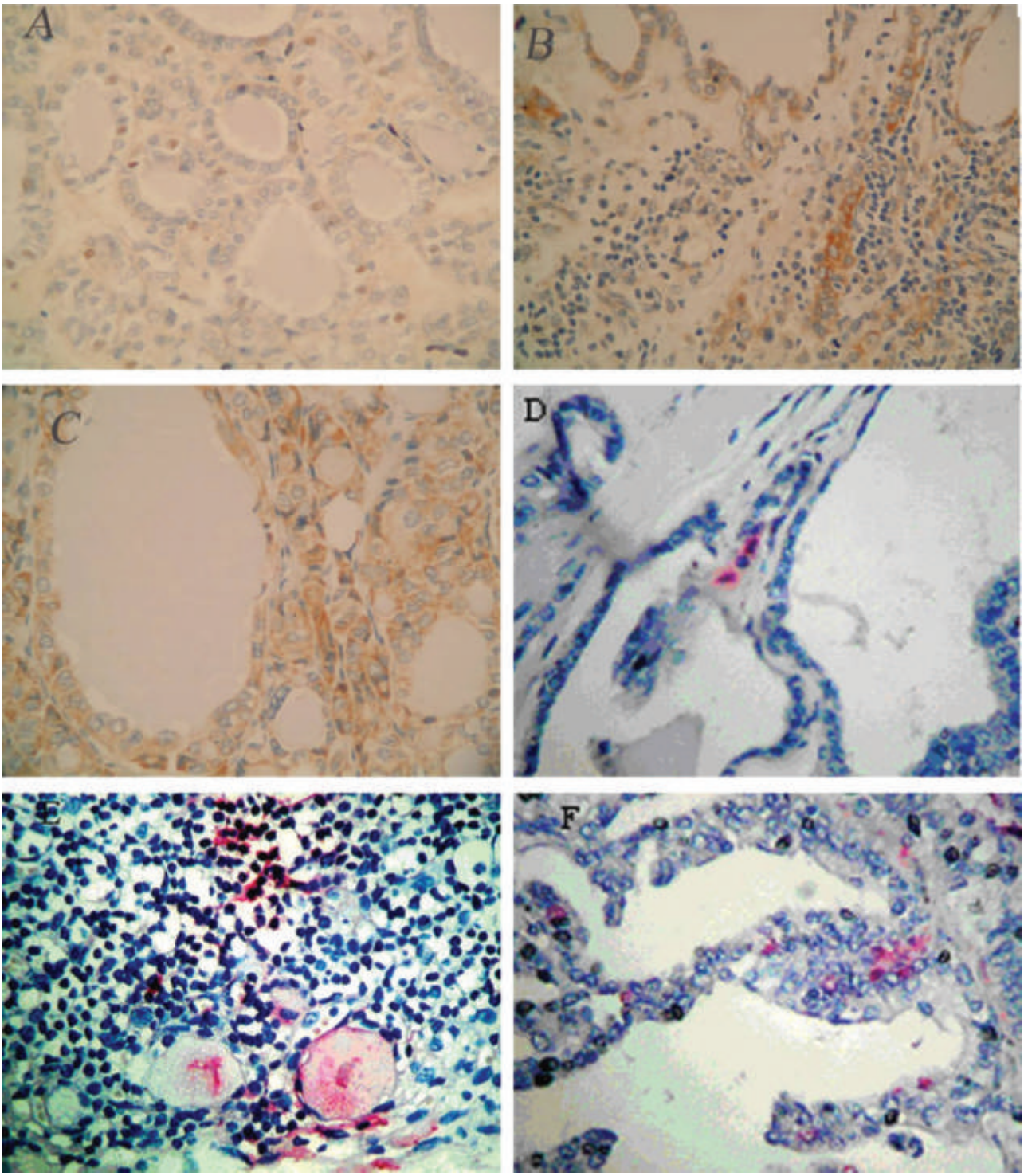

Figure 1. Expression of S-100 protein and CD83 in thyroid follicular adenoma, Hashimoto's thyroiditis and Graves' disease. Expression of S-100 protein was detected by immunohistochemical staining in TFA (A, ×400), HT (B, ×400) and GD (C, ×400) and those of CD83 in TFA (D, x400), HT (E, ×400) and GD (F, ×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).

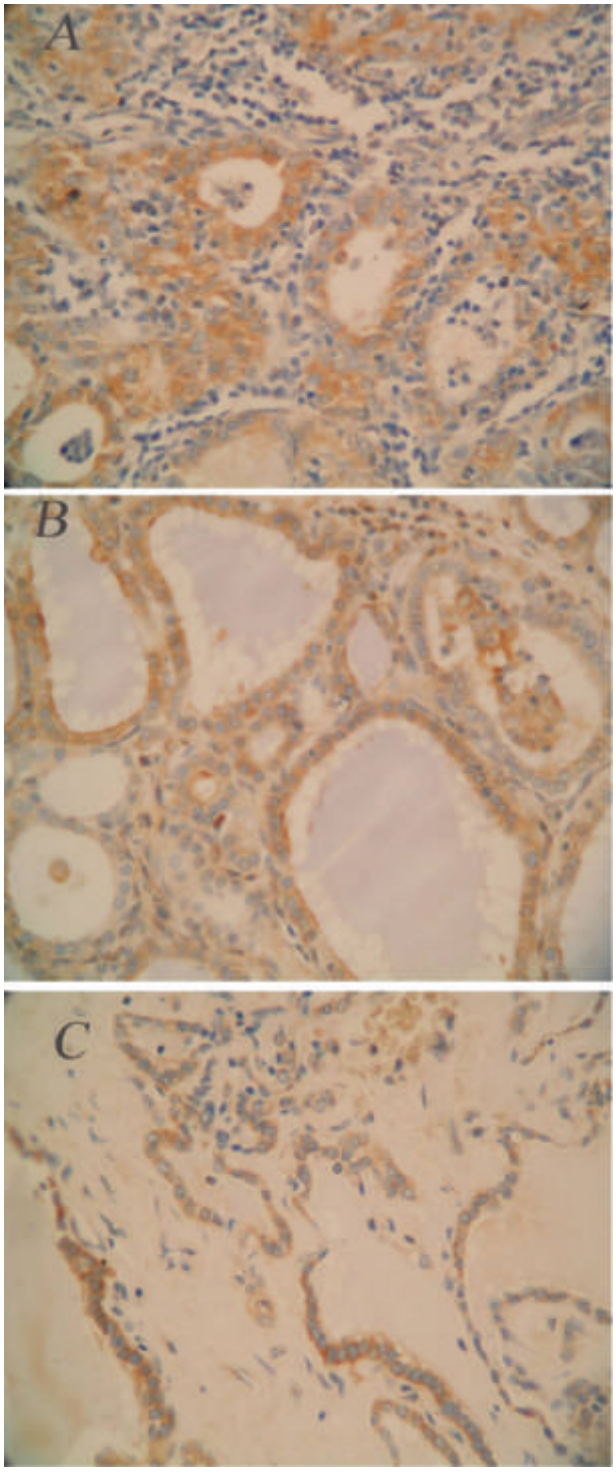

Figure 2. Representative staining pattern of Fas in Hashimoto's thyroiditis (A, ×400), in Graves' disease (B, ×400) and in thyroid follicular adenoma (C, ×100).

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.

Group	*n*	*Total prevalence (%)*	*Staining intensity (x±SD) (%)*
TFA	30	9/30 (30)	5.19±8.08
HT	30	23/30 (77)	22.58±13.96**
GD	30	24/30 (80)	29.92±14.43°°

HT *vs* TFA *p<0.05, **p<0.001<GD *vs* TFA °p<0.01, °°p<0.001.

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

	HT		*GD*		*TFA*
	Thyroid Follicles	*Lymphocytes*	*Thyroid Follicles*	*Lymphocytes*	
Fas	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)
FasL	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	83% (25/30)*
Bcl-2	73% (22/30)	63% (19/30)	97% (29/30)°°	97% (29/30)	83% (25/30)

**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.

	HT		*GD*		*TFA*
	Thyroid Follicles	*Lymphocytes*	*Thyroid Follicles*	*Lymphocytes*	
Fas	76.31±15.79**	43.42±22.85**	54.26±22.53	68.42±22.85	35.75±12.89
FasL	60.25±26.51*	46.10±25.80*	50.10±28.36	60.10±26.10	26.08±20.73
Bcl-2	6.75±10.40**#	12.78±11.39 **	14.30±17.20	6.17±16.73	14.80±21.26

** 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 percantages 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

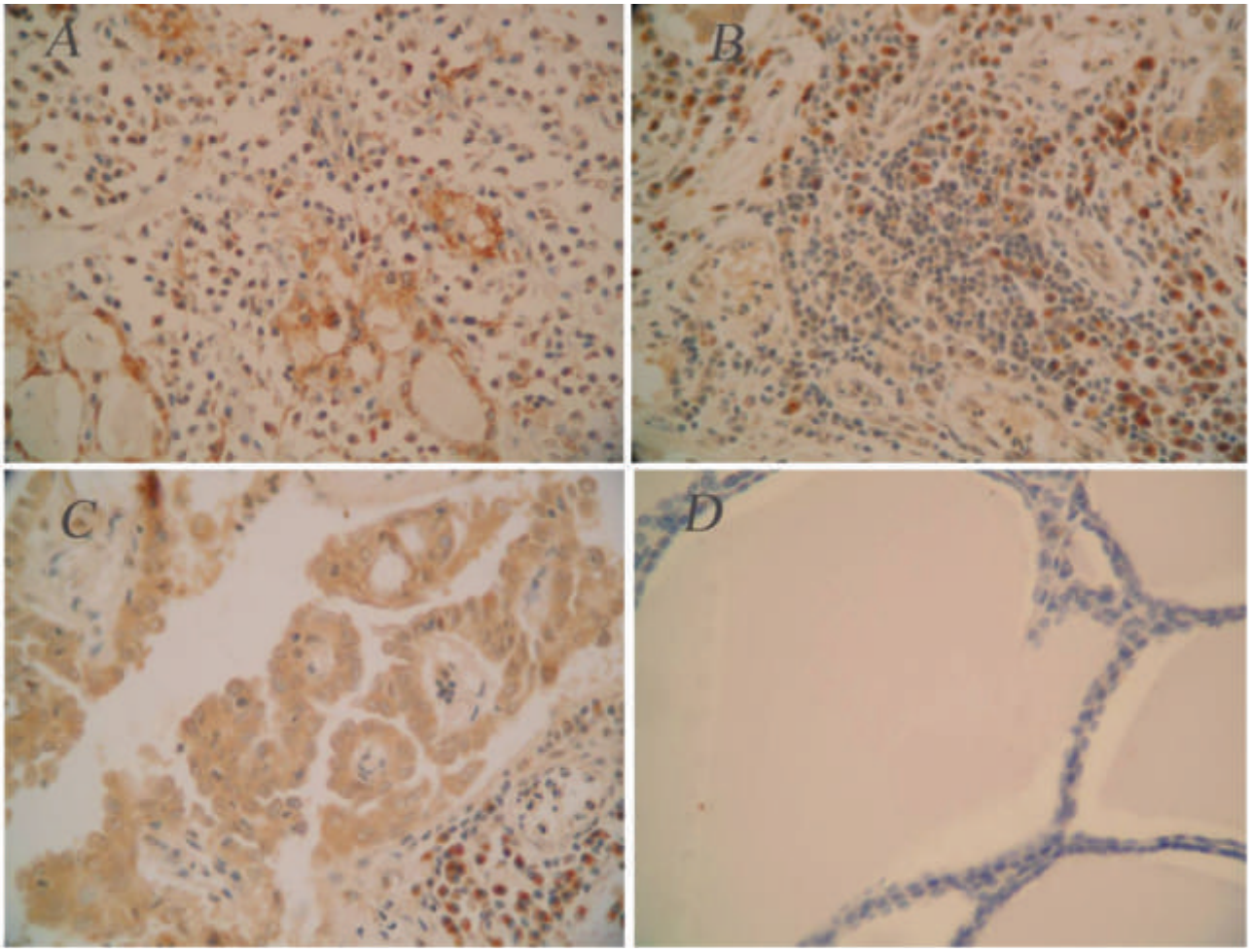

Figure 3. Representative staining pattern of FasL in thyroid follicles (A, ×400) and lymphocytes (B, ×400) of Hashimoto's thyroiditis and in Graves' disease (C, ×400). Note that it is negative in thyroid follicular adenoma (D, ×100).

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).

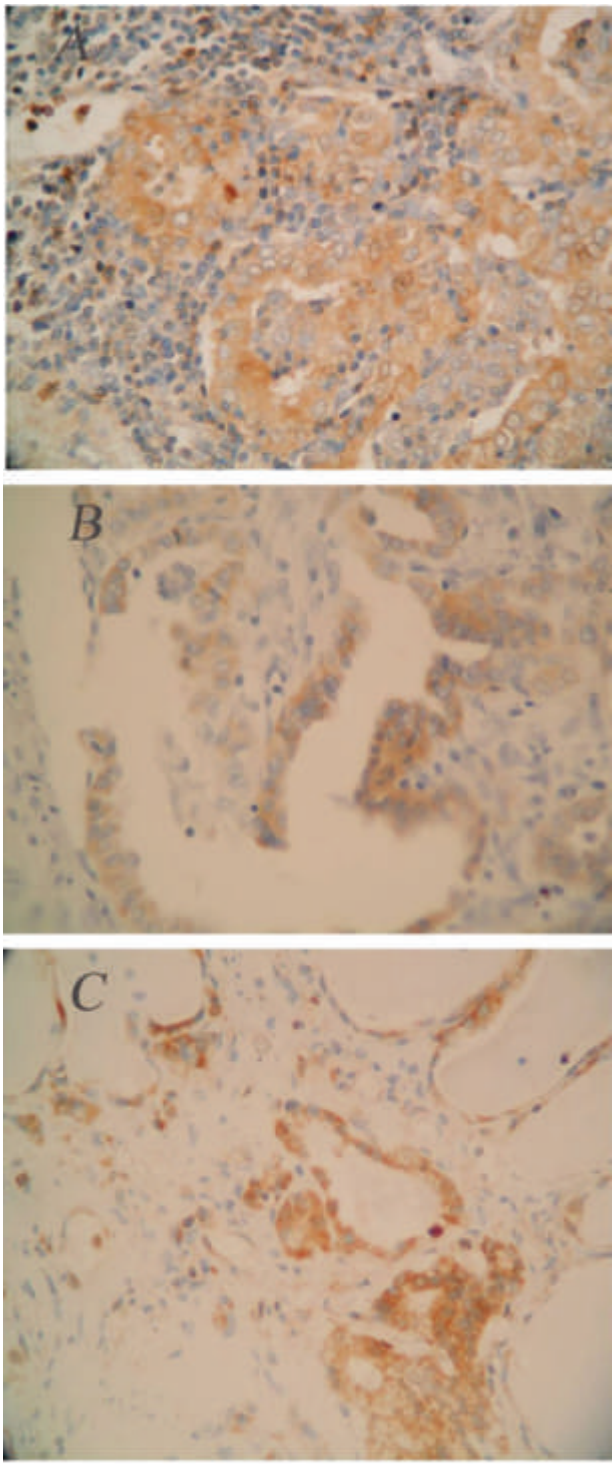

Figure 4. Representative staining pattern of Bcl-2 in Hashimoto's thyroiditis (A, ×400), in Graves' disease (B, ×200) and in thyroid follicular adenoma (C, ×200).

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

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 hypothyrodism 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 hyperthyrodism. 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 thyroids. 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

Arscott PL, Knapp J, Rymaszewski M, Bartron JL, Bretz JD, Thompson NW, et al. Fas(APO-1,CD95)mediated apoptosis in thyroid cells is regulated by a labile protein inhibitor. Endocrinology 1997; 138:5019-27.

Bagriacik EU, Klein JR. The thyrotropin (thyroid-stimulating hormone) receptor is expressed on murine dendritic cells and on a subset of CD45Rbhigh lymph node T cells :Functional role for thyroid-stimulating hormone during immune activation. J Immunol 2000; 164:6158-65.

Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998; 392:245-52.

Braley-Mullen H, Sharp GC. A thyroxine-containing thyroglobulin peptide induces both lymphocytic and granulomatous form of experimental autoimmune thyroiditis. J Autoimmun 1997; 10: 531-40.

Budd RC, Yeh WC, Tschopp J. cFLIP regulation of lymphocyte activation and development. Nat Rev Immuno 2006;6:196-204.

Canning MO, Ruwhof C, Drexhage HA. Aberrancies in antigen-presenting cells and T cells in autoimmune thyroid disease. A role in faulty tolerance induction. Autoimmunity 2003; 36:429-42.

Charles Sia, Arno Hänninen. Apoptosis in autoimmune diabetes: the fate of β-cells in the cleft between life and death rev diabet stud. 2006 Spring; 3:39-46.

Chen S, Akbar SM, Tanimoto K, Ninomiya T, Iuchi H, Michitaka K, et al. Absence of CD83 positive mature and activated dendritic cells at cancer nodules from patients with hepatocellular carcinoma:relevance to hepatocarcinogenesis. Cancer Letters 2000;148:49-57.

Cory S. Regulation of lymphocyte survival by Bcl-2 gene family. Annu Rev Immunol 1995; 13:513-43.

Delemarre FGA, Hoogeveen PG, de Haan-Meuknan M, Simons PJ, Drexhage HA. Homotypic cluster formation of dendritic cells. A close correlate of their state of maturation. Defects in the biobreeding diabetes-prone rat. J Leukoc Biol 2001;69:373-80.

Drakesmith H, Chain B, Beverley P. How can dendritic cells cause autoimmune disease? Immunol Today 2000;21:214-7.

Drexhage HA, Delemarre FGA, Radosevic K, Leenen PJM. Dendritic cells in autoimmunity. In: Lotze MT,Thomson AM (eds) Dendritic Cells. Biology and Clin Appl Academic Press, San Diego, California, 1999; pp 361-401.

Gallucci S, Matzinger P. Danger signals: SOS to the immune system. Curr Opin Immunol 2001; 13:114-9.

Giordano C, Stassi G, De Maria R, Todaro M, Richiusa P, Papoff G,et al. Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. Science 1997; 275:960-3.

Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA. Fas ligand-induced apoptosis as a mechanism of immune privilege. Science 1995; 270:1189.

Hawiger D, Inaba K, Dorsett Y. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J Exp Med 2001; 194:769-79.

Hiromatsu Y, Hoshino T, Yagita H, Koga M,Sakisaka S,Honda J,et al. Functional Fas ligand expression in thyrocytes from patients with Graves' disease. J Clin Endocrinol Metab 1999; 84:2896-902.

Kabel PJ, Voorbij HAM, de Haan M, van der Gaag RD,Drexhage HA. Intrathyroidal dendritic cells. J Clin Endocrinol Metab 1988; 66: 199-207.

Li HS, Verginis P, Carayanniotis G. Maturation of dendritic cells by necrotic thyrocytes facilitates induction of experimental autoimmune thyroiditis. Clin Exp Immunol 2006;144:467-74.

Lutz M, Schuler G. Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? Trends Immunol 2002; 23:445-9.

Nagata S, Golstein P. The Fas death factor. Science 1995; 267:1449-56.

Naqayama Y. Animal models of Graves' hyperthyroidism.Endocr J 2005;52:385-94.

Ninomiya T, Akbar SM, Masumoto T, Onji M. Dendritic cells with immature phenotype and defective function in peripheral blood from patients with hepatocellular carcinoma. J Hepatol 1999;31:323-31.

Mahiou J, Walter U, Lepault F, Godeau F, Bach JF, Chatenoud L. In vivo blockade of the Fas-Fas ligand pathway inhibits cyclophosphamide-induced diabetes in NOD mice. J Autoimmune 2001;16:431-40.

Marinkovic T, Garin A, Yokota Y, Fu YX, Ruddle NH, Furtado GC, et al. Interaction of mature $CD3^+CD4^+$ T cells with dendritic cells triggers the development of tertiary lymphoid structures in the thyroid. J Clin Invest 2006; 116: 2622-32.

Mitsiades N, Poulaki V, Kotoula V, Mastorakos G, Tseleni-Batafouta S, Koutras DA, et al. Fas/FasL ligand up-regulation and Bcl-2 down-regulation may be significant in the pathogenesis of Hashimoto's thyroiditis. J Clin Endocrinol Metab 1998; 83:2199-203.

Mizutori Y, Saitoh O, Eguchi K, Nagayama Y. Adenovirus encoding the thyrotropin receptor A-subunit improves the efficacy of dendritic cell-induced Graves' hyperthyroidism in mice. J Autoimmun 2006;26:32-6.

Mule JJ. Dendritic cells:at the clinical crossroads. J Clin Invest 2000;707-8.

Mysliwiec J,Okota M,Nikolajuk A,Gorska M.Soluble Fas, Fas ligand and Bcl-2 in autoimmune thyroid diseases: relation to humoral immune response markers. Adv Med Sci 2006;51:119-22.

Mysliwiec J, Oklota M, Nikolajuk A, Waligorski D, Gorska M. Serum CD40/CD40L system in Graves' disease and hashimoto's thyroiditis related to soluble Fas, FasL and humoral markers of autoimmune response. Immunol Invest 2007;36:247-57.

Pesce G, Fiorino N, Riccio AM, Montagna P, Torre G, Salmaso C, et al. Different intrathyroid expression of intercellular adhesion molecule-1 (ICAM-1) in Hashimoto's thyroiditis and Graves' disease: analysis at mRNA level and association with B7.1 costimulatory molecule. Endocrinol-Invest 2002; 25: 289-95.

Peters JH, Gieseler R, Thiele B, Steinbach F. Dendritic cells: from ontogenetic orphans to myelomonocytic descendants. Immunol Today 1996;17:273-8.

Quadbeck B, Eckstein SK, Tews S, Walz M, Hoermann R, Mann K,et al. Maturation of thyroidal dendritic cells in Graves' disease. Scand J Immunol 2002; 55:612-20.

Semra YK, Seidi OA, Sharief MK. Overexpression of the apoptosis inhibitor FLIP in T cells correlates with disease activity in multiple sclerosis. J Neuroimmunol 2001;113:268-74

Simons PJ, Delemarre FGA, Jeucken PHM, Drexhage HA. Pre-autoimmune thyroid abnormalities in the biobreeding diabetes-prone(BB-DP) rat: A possible relation with the intrathyroid accumulation of dendritic cells and the initiations of the thyroid autoimmune response. J Endocrinol 1998a; 157:43-51.

Simons PJ, Delemarre FG, Drexhage HA. Antigen-presenting dendritic cells as regulators of the growth of thyrocytes: A role for interleukin-1 beta and interleukin-6. J Endocrinology 1998b;139: 3148-56.

Simons PJ, Delemarre FG, Drexhage HA. A functional and phenotypic study on immuneaccessory cells isolated from the thyroids of wistar and autoimmune prone BB DP rats. J Autoimmun 2000; 15:417-24.

Stassi G, De Maria R. Autoimmune thyroid disease: new models of cell death in autoimmunity. Nat Rev Immunol 2002;2:195-204.

Suvannavejh GC, Dal Canto MC, Matis LA, Miller SD. Fas-mediated apoptosis in clinical remissions of relapsing experimental autoimmune encephalomyelitis. J Clin Invest 2000;105:223-31.

Suzuki A, Matsuzawa A, Iguchi T. Down-regulation of Bcl-2 is the first step on Fas-mediated apoptosis of male reproductive tract. Oncogene 1996;13:31-7.

Thome M, Tschopp J. Regulation of lymphocyte proliferation and death by FLIP. Nat Rev Immunol 2001;1:50-8.

Turley SJ. Dendritic cells: inciting and inhibiting autoimmunity. Curr Opin Immunol 2002; 14:765-70.

Veeraswamy RK, Cella M, Colonna M, Unanue ER. Dendritic cells process and present antigens across a range of maturation states. J Immunol 2003; 170:5367-72.

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.

Wei Y, Chen K, Sharp GC, Braley-Mullen H. Fas ligand is required for resolution of granulomatous experimental autoimmune thyroiditis. J Immunol 2004;173:7615-21.

Weir CR, Nicolson K, Backstrom BT. Experimental autoimmune encephalomyelitis induction in naive mice by dendritic cells presenting a self-peptide. Immunol Cell Biol 2002; 80:14-20.

Zhang Huijuan, Bei Yuexin, Li Daoming. Study on the immunohistochemistry of the dendritic cells in autoimmune thyroid diseases. Chinese Journal of Internal Medicine 1996;76:314-5.

Zhang Zhenlin, LIN Bo, YU Luyang, GUO Lihe.CMV-hFasL transgenic mice prevent from experimental autoimmune thyroiditis. Chin Med J(Engl) 2005 Jul 5;11.