

The Expression and Distribution of S-100 Protein and CD83 in Thyroid Tissues of Autoimmune Thyroid Diseases

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To investigate the expression and distribution of S-100 protein and CD83 in the thyroid tissues of autoimmune thyroid diseases (ATDs), and to study the role of the dendritic cells in the pathogenesis of ATDs, immunohistochemical staining was used on pathological tissues of 20 patients with Hashimoto's thyroiditis (HT) and 20 patients with Graves' disease (GD) to check the expression and distribution of S-100 protein and CD83. Compared with control group (20 cases of thyroid follicular adenoma, TFA), the higher expressions of S-100 in HT (139.38 ± 5.92 vs 59.47 ± 11.69) and GD (119.42 ± 14.48 vs 59.47 ± 11.69) were observed respectively ($p < 0.001$). The increased positive expressions of CD83 which is known as a marker of mature and activated DCs in HT (22.58 ± 13.96 vs 5.19 ± 8.08) and GD (29.92 ± 14.43 vs 5.19 ± 8.08) were also found respectively ($p < 0.001$). Serum TPO antibody (TPO-Ab, $67.3 \pm 11.6\%$) and Tg antibody (Tg-Ab, $59.8 \pm 10.1\%$) in HT were higher than that in GD ($28.4 \pm 5.7\%$, $23.1 \pm 4.9\%$) and that in TFA ($6.1 \pm 3.4\%$, $7.2 \pm 4.6\%$) ($p < 0.01$). Serum TR-Ab in GD (16.3 ± 5.6 U/L) was higher than that in HT (4.8 ± 2.3 U/L) and that in TFA (2.5 ± 1.2 U/L) ($p < 0.01$). Our findings suggest that the high expression of DCs' markers may be related to the pathogenesis of HT and GD. The upregulation of both number and matured functions of DCs, may lead to present more antigens and to produce more auto-antibodies (such as TgAb and TPOAb in HT, TRAb in GD), which may be involved in pathogenesis of the autoimmune thyroid diseases. *Cellular & Molecular Immunology*. 2004; 1(5):378-382.

Key Words: HT, GD, ATD, S-100 protein, CD83, immunohistochemistry

Introduction

With the advance of immunology, we know that the human immune system has the capability to recognize, process, present and destroy antigens. The immune system also plays a key role in the onset and development of autoimmune thyroid diseases (ATDs). Dendritic cells (DCs) are the most potent antigen presenting cells (APCs) and are able to induce primary immune responses both *in vitro* and *in vivo* (1). DCs have the capability to capture, process and present self-antigen and induce autoimmune diseases (2, 3). T cell-mediated autoimmune is the result of inappropriate self-antigen presentation (4). Self antigens that have entered the endocytic pathway of the APCs are processed there and generally presented by MHC-II molecules to T

cells. DCs are specialized APCs which are able to present antigens to naive and quiescent T cells and consequently play a control role not only in initiation but also in the maintenance of ATDs. Mature DCs (CD83 positive) can effectively stimulate B cells which can be motivated and differentiated, produce antibodies, and then the antibodies can induce inflammatory reaction of the thyroid tissue. DCs also have the ability to produce cytokines and chemokines which can attract lymphocytes and monocytes to inflammatory tissues. Nowadays, it is known that S-100 protein is a non-special marker of DCs and CD83 antigen is a special marker of mature, activated human DCs (5). In this study, we tried to use immunohistochemical method to study the expressions and distributions of S-100 protein and CD83 in thyroid tissues of different ATDs, to get some information about the role of DCs in the pathogenesis of them.

Materials and Methods

Subjects and thyroid tissues

20 patients with HT (female, 31~63 years) and 20 patients with GD (female, 28~65 years) were enrolled in this study. Normal thyroid tissues adjacent to thyroid follicular adenoma (TFA) obtained from 20 subjects with TFA

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Received for publication Aug 31 2004. Accepted for publication Sep 26, 2004.

(female, aged 26~60 years) were assigned as control. All patients admitted to the First Hospital of Shantou University Medical College between 2001 and 2002 were diagnosed according to the clinical conditions and evaluation of data of the appropriate tests, and were confirmed by histological examination (hematoxylin and eosin staining) of the thyroid tissue samples. Informed consent was taken from all patients and control subjects after explaining the nature and purpose of the study. Thyroid tissue specimens for this study were collected during surgical operation. All samples were fixed in 10% buffered formalin, embedded in paraffin, and cut into 4 μ m sections.

TPO-Ab, Tg-Ab and TRAb checking

Serum of peripheral blood from every patient was collected and checked for TPO-Ab, Tg-Ab and TRAb by radioimmunoassay (RIA) method (radioimmunoassay kit, Beijing North Institute of Biological Technology, Beijing, China).

Immunohistochemical staining

Immunohistochemical staining SP method was performed to detect S-100 protein, rabbit anti-human polyclonal antibody to S-100 protein (PharMingen International, CA) was used as primary antibody, and diaminobenzidine (DAB)-hydrogen peroxide was employed as chromogen. The staining of CD83 was performed with some modifications according to a previous method (5, 6). Formalin-fixed, paraffin-embedded thyroid specimens were washed three times in phosphate buffered saline (PBS) and treated with pepsin (0.5% in 0.01 N 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. Appropriate dilution (1:100) of mouse anti-human monoclonal antibody as primary antibody (PharMingen, San Diego, CA) was then added and incubated over night. The sections were then washed with PBS three times and reincubated with biotinylated goat anti-mouse immunoglobulin (1:200, DAKO, Denmark) at room temperature for 1 h. After a wash with PBS, sections were soaked in alkaline phosphatase-conjugated streptavidin (DAKO), washed, and New Fuchshin (DAKO) were 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 and negative control slides were processed with PBS liquid instead of the primary antibody, but included all other steps of the procedure.

Positive staining estimation

Expression of S-100 protein was located on nucleus and/or cytoplasm of DCs, appearing brown granules and the expression levels were stronger than background staining. Expression of CD83 showing red granules was located on membrane and/or cytoplasm of DCs, CD83 positive cells were counted over the whole specimens. The intensities of positive staining of S-100 was calculated by HpIAS1000 analysis system, shown as AGV (average grey value = [average positive grey value – average background grey value]), the frequencies of CD83 positive cells was shown as total numbers of cells/specimen.

Table 1. Levels of TPO-Ab, Tg-Ab and TRAb in TFA, HT and GD.

Group	Number	TPO-Ab (%)	Tg-Ab (%)	TRAb (U/L)
TFA	20	6.1 \pm 3.4	7.2 \pm 4.6	2.5 \pm 1.2
HT	20	67.3 \pm 11.6*	59.8 \pm 10.1*	4.8 \pm 2.3
GD	20	28.4 \pm 5.7	23.1 \pm 4.9	16.3 \pm 5.6**

*HT compared with GD and TFA respectively, $p < 0.01$; **GD compared with HT and TFA respectively, $p < 0.01$.

Statistical analysis

The data were expressed as mean \pm SD. The statistical analysis was done by unpaired or paired t -tests. Positive rates of S-100 protein and CD83 in TA, HT and GD were shown as %, the data analysis were made with χ^2 test. P -value less than 0.05 was considered to indicate statistical significance. Statistical calculations were performed using the SPSS 10.0 statistical program.

Results

The level of TPO-Ab, Tg-Ab and TRAb

Serum TPO antibody (TPO-Ab) and Tg antibody (Tg-Ab) in HT were respectively higher than that in GD and that in TFA ($p < 0.001$). Serum TRAb in GD was higher than that in HT and that in TFA ($p < 0.001$). See Table 1.

The expression of S-100 protein

The distribution of S-100 protein positive DCs were close contact with thyroid follicular cells or located in infiltrating lymphocytes, it was detected in the nucleus and cytoplasm, S-100 protein was positive in all thyroid tissues, positive rates of three groups were all 100%. However, TFA tissue seldom expressed S-100 protein (with staining intensity 59.47 \pm 11.69, Figure 1A). The expression of S-100 protein in HT and GD were elevated (with staining intensity 139.38 \pm 5.92 and 119.42 \pm 14.48 respectively (Figure 1B, C) (Table 2).

The expression of CD83

The CD83 positive DCs were also distributed in infiltrating lymphocytes, expressed in the cytoplasm. CD83 seldom expressed in TFA tissues (with positive rate of 30%, staining intensity 5.19 \pm 8.08, Figure 1D), but more expressed in HT (with positive rate of 75%, staining

Table 2. S-100 protein expression in group of TFA, HT and GD.

Group	Number	Positive rate (%)	Staining intensity (x \pm SD)
TFA	20	100	59.47 \pm 11.69
HT	20	100	139.38 \pm 5.92*
GD	20	100	119.42 \pm 14.48**

*HT compared with TFA, $p < 0.001$; **GD compared with TFA, $p < 0.001$.

Table 3. CD83 expression in group of TFA, HT and GD.

Group	Number	Positive rate (%)	Staining intensity ($\bar{x} \pm SD$)
TFA	20	6/20 (30)	5.19 \pm 8.08
HT	20	15/20 (75)*	22.58 \pm 13.96**
GD	20	16/20 (80) [#]	29.92 \pm 14.43 ^{##}

HT compared with TFA, * $p < 0.05$, ** $p < 0.001$; GD compared with TFA, [#] $p < 0.01$, ^{##} $p < 0.001$.

intensity 22.58 ± 13.96 , Figure 1E) and GD (with positive rate of 80%, staining intensity 29.92 ± 14.43 , Figure 1F), higher than that in TFA ($p < 0.05$) (Table 3).

Discussion

The pathogenesis of autoimmune thyroiditis has been studied in detail in the animal model that spontaneously develop autoimmune thyroiditis (7). The studies of the animal model have shown that the pathogenesis of the autoimmune failure of the thyroid is a multistep process, requiring several genetic and environmental abnormalities (or variants) to converge before full-blown disease develops.

At the early phases of the disease, two immune processes can be distinguished. Firstly, the afferent phase is characterized by an intra-thyroid accumulation of DCs. Blood monocytes are an important precursor population of these cells and able to mature into various subsets of DCs (8). DCs are the antigen-presenting cells (APCs) par excellence, and essential for stimulation of naïve T cells, leading to sensitization and clonal expansion of the latter (9). Increased numbers of major histocompatibility complex (MHC) class II-positive DCs have been found both inside and outside lymphocytic accumulations in the thyroids of patients with Graves' disease or Hashimoto's goiter (10),

and in the thyroids of the animal model (11, 12). In the 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 (13, 14). The fate of DCs accumulated in tissues is to enter the lymphatics to travel to the draining lymph nodes while transporting antigens. Homotypic interactions play a role in activation and further maturation of DCs (15).

What attracts these DCs to the thyroid in these prodromal phases? First, attraction signals will produce in nature. Early unspecific 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 in defense or 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* (16) via IL-1 and IL-6 (17). Moreover, DCs are responsive to TSH (because they express the TSH receptor) and to thyroid hormones, and particularly produce proinflammatory cytokines such as IL-1 β and IL-12 under such conditions (18-20).

On the other hand, as a consequence, 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 of autoantibodies of the immunoglobulin G (IgG) class. Initially this production of autoreactive cells and autoantibodies take place in the draining lymph nodes. Later, however, lymphoid tissue often develops locally in the thyroid gland of the BB-DP rat (1). 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 anti-thyroglobulin (anti-Tg) antibodies.

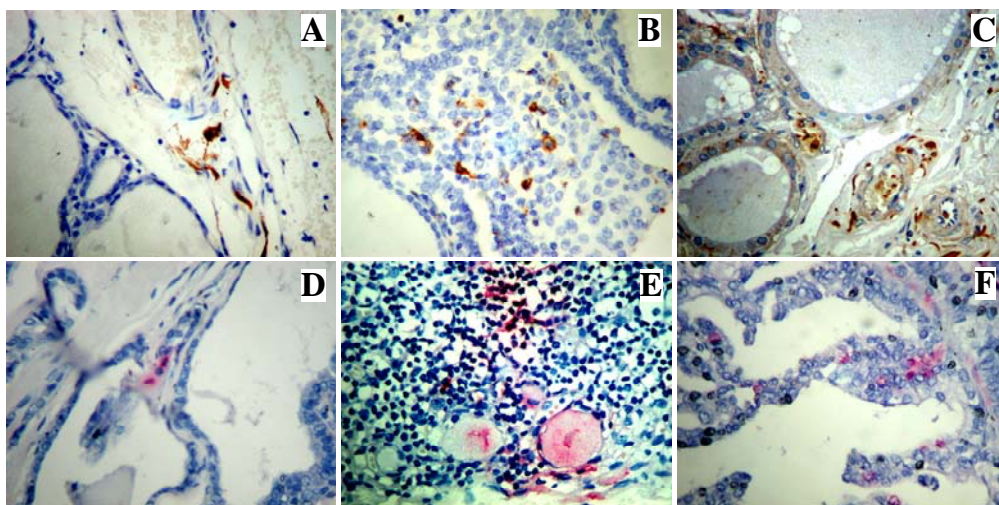


Figure 1. Expression of S-100 protein and CD83 in TFA, HT and GD. The expressions of S-100 protein were detected by immunohistochemical staining in TFA (A, 400 \times), HT (B, 400 \times) and GD (C, 400 \times) and those of CD83 in TFA (D, 400 \times), HT (E, 400 \times) and GD (F, 400 \times).

Our data showed that S-100 protein positive DCs and CD83 positive DCs accumulated in the thyroid tissues of HT and GD patient, these DCs were in close contact with thyroid follicular cells or located in infiltrating lymphocytes. These show 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 positive DCs are considered as mature and activated dendritic cells which also can express high level of co-stimulatory factors (such as CD80 and CD86), these CD83 positive DCs have very powerful antigen presenting ability (21). Studies (22-24) have showed 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 huijiang (25) had discovered that the extent of infiltration of DCs in the thyroid tissues of HT was highly positive correlated with the value of TgAb and TPO-Ab. This suggests that the autoimmune failure of the thyroid and the high level of autoantibodies are correlated with the increase number of DCs. Our study showed that the number of CD83 positive DCs was increased in the thyroid tissues of HT and GD, but the thyroid tissues of TFA seldom express CD83. Our findings are consistent with others (22-25).

In HT, the autoreactive T cells diffusely accumulate in large numbers and infiltrate the thyroid parenchyma. It has been shown in the BB-DP rat model that Th1 type cytokines such as IL-12, tumor necrosis factor- α (TNF- α), IL-2, and interferon- γ (IFN- γ) play a major role, rather than Th2 type cytokines (IL-4, IL-10) (26). The infiltration of activated scavenger macrophages into the thyroid follicles destroying the thyroid follicles is compatible with such view (12). Fas and FasL expression was also higher in rats and human with lymphocytic thyroiditis, indicating a role of these apoptotic molecules in thyrocyte death (27, 28).

In GD, thyrotropin (TSH)-receptor antibodies play a prominent role in the effector phase. These autoreactive receptor antibodies (and perhaps other immune components) induce an excessive growth/metabolic stimulation of the target tissue respectively.

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