Antigenic determinants on thyroglobulin 
recognized by T cells 
in patients with chronic thyroiditis

Naoki Shimojo¹, Yoichi Kohno¹, Osamu Tarutani²,
Nozomu Sasaki³ and Hironori Nakajima¹

Department of Pediatrics¹, School of Medicine, Chiba University, Chiba, Japan;
Department of Physical Biochemistry², Institute of Endocrinology, Gunma University, Maebashi, Japan

Abstract. It was found that the proliferating cells to thyroglobulin (Tg) in patients with chronic thyroiditis are confined to a T cell subpopulation. Then, we attempted to characterize the antigenic determinants of Tg recognized by T cells from patients with chronic thyroiditis by Tg-induced DNA-synthetic response. T cells from patients could respond to Tgs from whales, pigs and chickens as well as to human Tg. These findings indicated that epitopes on Tg recognized by T cells were shared by Tgs from various species. In addition, the T cells from different individuals varied in specificity.

Autoimmunity to thyroglobulin (Tg) in patients with chronic thyroiditis has been studied for the last decade. However, the mechanism of induction of chronic thyroiditis is still unknown. The idea that T cells exert a critical role in the regulation of autoimmunity to Tg has received considerable support from several groups (Weigle 1980; Roitt et al. 1980; Rose et al. 1981). Peripheral lymphocytes from patients with chronic thyroiditis exhibit a marked DNA-synthetic response to Tg in vitro (Calder & Irvine 1975; Aoki & DeGroot 1979; Canonica et al. 1984). Furthermore, some investigators (Blomgren & Lundell 1979; Canonica et al. 1984) have reported that the DNA-synthetic response triggered by Tg was not due to a non-specific stimulatory effect of thyroid hormones, but rather to antigen stimulation of the cells, and that T cells constituted the responding cells to Tg. As mentioned above, blastogenic response to Tg in vitro is a good tool for studying the role of T cells in autoimmunity to Tg.

Different antigenic determinants on multideterminant protein antigens may be recognized by different functional subsets of specific immunocompetent cells (Sercarz et al. 1978). The antigenic determinants on the protein, Tg, provoking the autoimmunity especially recognized by T cells, have to be determined in order to clarify the pathophysiology of autoimmunity in thyroid diseases. To ascertain the role of antigenic determinants on Tg in regulating the autoimmune response to Tg, their cross-reactivities with epitopes of Tg from different species in T cell recognition were studied.

Materials and Methods

Subjects
Eight patients with chronic thyroiditis, denoted as P1 to P8 and ranging in age from 13 to 27 years (mean 17 years), were examined. Healthy persons, aged 26–32 years, without any known thyroid disorder served as controls. The diagnosis was made on the basis of clinical and laboratory data and histologically confirmed by needle biopsy in all the patients.

Preparation of thyroglobulins
Thyroglobulins from humans, whales or chickens were prepared using the techniques described previously.
(Kohno et al. 1985). Porcine Tg was obtained from Sigma Chemical Co.

**Cell culture**

Peripheral blood lymphocytes were collected from heparinized venous blood by centrifugation on Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden). They were washed three times with Hank's balanced salt solution (GIBCO, NY, USA), and finally suspended in RPMI 1640 (GIBCO) containing 10% heat-inactivated pooled human serum, 100 000 U/l of penicillin, 100 mg/l of streptomycin (GIBCO), 2 mM L-glutamine (GIBCO) and 50 μM 2-mercaptoethanol (Wako Pure Chemicals, Tokyo, Japan). Cell cultures were performed in triplicate in flat-bottomed microculture plates (Costar, Cambridge, MA, USA) at 37°C in 5% CO2 in moist air for 7 days, each well containing 2 x 10⁵ cells in 0.2 ml of culture medium with or without Tg at different dose levels (0.001–50 mg/l). Similarly, lymphocytes were cultured with purified protein derivative (PPD) (Japan BCG Products, Tokyo, Japan) at the concentration of 25 mg/l as an unrelated antigen. The cells were pulsed with 0.5 μCi of [3H] methylthymidine (New England Nuclear, Boston, MA, USA) (specific activity 6.7 Ci/mmol) for the final 16 h of culture. Cultured cells were harvested on a glass filter by cell harvester (Abe Kagaku, Chiba, Japan), and thymidine uptake was determined with a liquid scintillation counter (LKB Wallac, Turku, Finland). Thymidine uptake was expressed as counts per min (Δcpm), which were derived by subtracting the values of unstimulated cultures from antigen-stimulated ones. The arithmetic average of the mean cpm and the standard error (SEM) were calculated. Stimulation index (SI) by Tg or PPD was calculated for all culture groups as follows:

\[ SI = \frac{\text{cpm in Tg or PPD culture}}{\text{cpm in control culture without antigen}} \]

**Separation of lymphocyte subpopulation**

Using a rosette technique reported by Dean et al. (1975), subpopulations of lymphocytes were separated. The frequency of T cells in the preparations were determined by counting the proportion of cells stained by OKT3 or OKT11 antibody (Ortho Diagnostic Systems, Raritan, NJ, USA). Samples were analyzed by flow cytofluorometry (Spectrum III; Ortho Diagnostic Systems). The non-purified preparations contained 87% of OKT11 and 70% of OKT3 positive cells. In T cell enriched preparations, 95% of OKT11 and 76% of OKT3 positive cells were detected whereas 30% of OKT11 and 9% of OKT3 positive cells were found in T cells depleted preparations.

**Statistics**

Statistical analysis of the results was performed with Student's t-test. Differences were considered significant when \( P < 0.05 \).

Results are given as arithmetic mean ± 1 SEM.

---

**Fig. 1.**

[3H]thymidine incorporation in vitro in response to Tg. Lymphocytes from patient P1, with chronic thyroiditis and a healthy control were cultured with various concentrations of Tg, ranging between 0.001 and 50 mg/l. The values of unstimulated cultures of lymphocytes from P1 and the healthy control were 1455 ± 332 cpm and 1336 ± 207 cpm, respectively.
**Table 1.**  
Peripheral lymphocyte proliferation in vitro to Tgs from various species.

<table>
<thead>
<tr>
<th>Patient</th>
<th>[3H]thymidine uptake (Δ cpm ± SEM)</th>
<th>Thyroglobulin</th>
<th>PPD4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Human Tg</td>
<td>Whale Tg</td>
</tr>
<tr>
<td>P2</td>
<td>3425 ± 1075*</td>
<td>−296 ± 179</td>
<td>−805 ± 166</td>
</tr>
<tr>
<td></td>
<td>3.62( ^\text{1} )</td>
<td>0.77</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>(5)( ^\text{2} )</td>
<td>(0.01)</td>
<td>(10)</td>
</tr>
<tr>
<td>P3</td>
<td>2262 ± 169*</td>
<td>4528 ± 831*</td>
<td>736 ± 619</td>
</tr>
<tr>
<td></td>
<td>3.06</td>
<td>5.13</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>(0.05)</td>
<td>(0.1)</td>
<td>(10)</td>
</tr>
<tr>
<td>P4</td>
<td>4206 ± 577*</td>
<td>10070 ± 2528*</td>
<td>4819 ± 41*</td>
</tr>
<tr>
<td></td>
<td>2.80</td>
<td>3.72</td>
<td>2.30</td>
</tr>
<tr>
<td></td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(1)</td>
</tr>
<tr>
<td>P5</td>
<td>11187 ± 82*</td>
<td>8996 ± 368*</td>
<td>N.T.3</td>
</tr>
<tr>
<td></td>
<td>3.62</td>
<td>3.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.001)</td>
<td>(0.005)</td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>19725 ± 3117*</td>
<td>11390 ± 2998*</td>
<td>N.T.</td>
</tr>
<tr>
<td></td>
<td>7.15</td>
<td>4.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.005)</td>
<td>(0.005)</td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>8720 ± 2691*</td>
<td>1433 ± 1128</td>
<td>N.T.</td>
</tr>
<tr>
<td></td>
<td>3.99</td>
<td>1.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(10)</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from the response to Tg in healthy control at the concentration of Tgs indicated in each response. The responses to Tgs in healthy controls used in Experiment 1 and 2 are shown in Fig. 2.

1 Stimulation index.

2 Numbers in parenthesis represent Tg concentration (mg/l) at the maximum response obtained.

3 N.T.: not tested.

4 PPD was used at the concentration of 25 mg/l as an unrelated antigen.

**Results**

**Antigen concentration in the culture**

Optimal proliferative response was obtained by culturing lymphocytes for 7 days at 2 \times 10^5 cells. The response to Tg at 2 \times 10^5 lymphocytes with different dose levels (0.001–50 mg/l) on day 7 of culture was examined. As shown in Fig. 1, lymphocytes from P1 responded to human Tg at the concentration of 0.005, 1 or 50 mg/l. In a preliminary study, it was found that a Tg concentration of 50 mg/l or higher in cultures induced a non-specific response in some normal subjects. Therefore, it was suggested that two dose levels, i.e., 0.005 and 1 mg/l of Tg, were optimal for blastogenesis in this case. In addition, the optimal dose level of Tg for blastogenesis differed from patient to patient (Table 1). Thus, it is not possible to set a single dose level of Tg for evaluation of Tg-induced blastogenesis. Therefore, various concentrations of Tg (0.001–10 mg/l) were studied in each lymphocyte proliferative response. Furthermore, lymphocytes from five healthy persons tested did not show any response to various Tgs at any concentrations of Tg (Figs. 1 and 2).
[\textsuperscript{3}H]thymidine incorporation in vitro into lymphocytes from patients with chronic thyroiditis in response to Tgs from various species. Mean values for responses of lymphocytes from two healthy subjects to Tg are shown in Experiment 1 and 2. •——• Patient with chronic thyroiditis; •———• Healthy control. A) P3 and healthy control in Experiment 1; B) P6 and healthy control in Experiment 2. Data represent mean \( \Delta \text{cpm} \pm \text{SEM} \) of triplicate samples. The values of unstimulated cultures of lymphocytes from P3, P6 and a healthy control in panel A and a healthy control in panel B were 1094 ± 185 cpm, 3204 ± 668 cpm, 1468 ± 241 cpm and 2897 ± 268 cpm, respectively.

**Identification of the lymphocyte subpopulation responding to Tg**

To study which cell subpopulation was responsible for the proliferative response to Tg, the lymphocytes were fractionated into T cell enriched and T cell depleted cells. No significant proliferative response to Tg in T cell depleted preparations from patients was observed, whereas the response in T cell enriched preparations was similar to that of unfractionated cells (Table 2). Therefore, the proliferating cells to Tg in patients might be confined to a T cell subpopulation.
Response to heterologous thyroglobulin

We compared the responsiveness of lymphocytes from patients with chronic thyroiditis to Tgs from various species, i.e. humans, chickens, whales or pigs, in order to ascertain the antigenic determinants on the Tg molecule recognized by T cells (Fig. 2, Table 1). P3 and P6 are shown in Fig. 2 as typical examples of the results of responses to various Tgs in patients with chronic thyroiditis. Summarized data for the Tg-induced blastogenesis assay are shown in Table 1. In the case of P3, lymphocytes demonstrated a response to whale Tg as well as to human Tg, but not to Tgs from chickens and pigs. P4 showed a positive response to Tgs from humans, whales and pigs, but not to chicken Tg. In the cases of P5 and P6, responses to Tgs from humans, whales and chickens were observed. Lymphocytes from P2 and P7 responded only to human Tg among the various Tgs used in each study. These patients, P2 and P7, gave a good proliferative response to PPD and the values of the stimulation index for human Tg-induced blastogenesis were not lower in these patients than those in the other patients. Therefore, the differences shown by lymphocytes from different individuals in response to Tgs from a variety of species could not be due to general differences in immune responsiveness. Taken together, these findings indicate that T cells responding to human Tg in patients with chronic thyroiditis cross-react with other mammalian Tgs. In addition, the specificities of T cells to human Tg from different individuals were heterogeneous.

Discussion

In order to study the antigenic determinants of Tg recognized by lymphocytes from patients with chronic thyroiditis, we developed an in vitro culture system by which the Tg-specific proliferative response could be measured. It has been reported that proliferating cells in patients with chronic thyroiditis are confined to a T cells subpopulation (Blomgren & Lundell 1979; Canonica et al. 1984). Our data provided evidence to support these results.

More than two optimal concentrations of human Tg for lymphocyte DNA synthesis were observed in our patients. More than one optimal dose response level of Tg for blastogenesis in culture suggested that two different clones of T cell responded to different epitopes on Tg. An alternative explanation may be that there are secondary binding sites of lower affinity on the Tg molecule, since the preliminary reports of the amino acid sequence of Tg indicate regions of similar amino acid structure which are repeated (Palumbo et al. 1983; Palumbo & Tecce 1984).

The globular protein, Tg, has multiple antigenic determinants on its molecule (Nye et al. 1980; Husby et al. 1983). The protein structure and function of Tg have not been elucidated owing to the large size of the protein. One way to distinguish the epitopes is to compare their cross-reactivity with epitopes of Tg from different species. Our previous data indicated that autologous antibodies to Tg cross-react with other mammalian Tgs (Kohno et al. 1985), and, as described in this paper, epitopes recognized by T cells are also shared with Tgs from various species. In other words, autoreactive antigenic determinants on human Tg recognized by T cells as well as by B cells are shared by various mammalian Tgs. In contrast, Baur & Goodman (1964) reported that anti-autologous Tg from the serum of a patient with chronic thyroiditis reacted only with human Tg or Tg from the Old World monkey, but did not cross-react with any other mammalian Tg.
On the other hand, experimental autoimmune thyroiditis in animals produced by immunization with aqueous preparations of various heterologous Tgs has been reported (Witebsky & Rose 1959; Weigle & Nakamura 1967; Weigle 1980; Romball & Weigle 1983). This suggested the existence of interspecies cross-reactive determinants of Tg recognized by B cells and/or T cells. Romball & Weigle (1984), however, reported that no lesions were observed in the thyroid glands of mice immunized with bovine Tg in complete Freund's adjuvant (CFA), whereas mice immunized with mouse Tg in CFA had extensive lesions in their thyroid glands, and that bovine Tg-primed T cells in mice did not cross-react with mouse Tg.

As suggested by Romball & Weigle (1984), the mechanism of induction of experimental autoimmune thyroiditis appears to vary dependent on the immunization protocol. In any case, they examined the cross-reactivities with other Tgs with antibodies or T cells induced as a result of injections of heterologous Tgs, but not those of antibodies of T cells induced spontaneously.

In conclusion, this work demonstrates that T cells responding to Tg from patients with chronic thyroiditis cross-react with Tgs from various mammalian species. In addition, the specificities of T cells to Tg in patients could be very heterogeneous. The understanding of such epitopes should provide additional insight into the mechanisms of thyroid autoimmunity.

References


Received on June 3rd, 1985.

Downloaded from biocatiantica.com at 11/12/2018 06:11:13PM via free access