Specific stimulatory effects of Graves' IgG on the release of triiodothyronine from the patients' own thyroids

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Abstract. The present study was undertaken to investigate the release of thyroid hormones from cultured thyroid slices of untreated patients with Graves' disease in response to autologous IgG and IgG from other untreated patients with Graves' disease. Thyroid tissue (8-10 mg) was obtained by needle biopsy from 11 untreated patients with Graves' disease, and each biopsy was divided into 5 slices. Slices were then cultured in Ham's F-12 synthetic media for 7 days with IgG (1.3 mg/ml) obtained from the same patients, IgG obtained from other untreated patients, normal IgG, or bovine TSH (1.3 mIU/ml). Media were changed every day, and the concentrations of triiodothyronine (T₃) in the media were measured by radioimmunoassay (RIA), and the concentrations of thyroidal cAMP were measured by radioimmunoassay on the last day of culture after incubation with 10 mM theophylline at 37°C for 30 min. When thyroid slices were incubated with autologous IgG, the release of T₃ increased from the 3rd day, and the increase was 3- to 10-fold above controls on the 5th day, and the production of thyroidal cAMP significantly increased 2- to 5-fold. However, when slices were incubated with IgG obtained from other untreated patients, the concentration of T₃ in media and the production of thyroidal cAMP did not differ from that in controls.

TSH increased the release of T₃ from thyroid slices 2.5- to 10-fold above controls on the 5th day and the production of cAMP 4- to 5-fold. These results strongly suggest that thyroid hormone releasing IgG in patients with Graves' disease are highly specific for autologous thyroids. The mechanism of this specificity may involve the role of self-recognition, such as antiidiotype antibody or anti-major histocompatibility complex antibody.

It has been reported from many laboratories that Graves' IgG inhibits the binding of TSH to thyroid plasma membrane and stimulates thyroidal adenylate cyclase activity (Burman & Baker 1985). However, the results obtained from various methods have not consistently correlated with the clinical course of Graves' disease (Kuzuya et al. 1979; Nagataki et al. 1980; Shishiba et al. 1982), and a dissociation has been reported with the utilization of different methods involving untreated as well as treated patients (Sugenoya et al. 1979; Kuzuya et al. 1979).

In order to determine the stimulatory effects of Graves' IgG, it seemed to us that the ideal method would be to use human thyroid tissue, to observe an end-product of stimulation, such as thyroid hormone release as an index, and to observe chronic stimulatory effects since thyroids of Graves' patients are being chronically stimulated. Recently we have developed a new technique of organ culture of human thyroids to measure the release of thyroid hormones while being stimulated chronically by TSH (Nagataki 1982; Hamada et al. 1983). However, in spite of a significant increase in thyroidal cAMP concentrations, the release of thyroid hormones from normal thyroid tissues was not stimulated by Graves' IgG (Nagataki 1982). Since the release of thyroid hormones from thyroids of patients with Graves'
disease must be stimulated by their own IgG, we felt that the stimulatory effects of IgG could be observed if it were incubated with autologous thyroids in this organ culture system.

The present experiments were undertaken, therefore, to investigate the release of thyroid hormones from cultured thyroid slices of untreated Graves' patients in response to their own IgG and to IgG from other untreated patients with Graves' disease.

Materials and Methods

Patients (Table 1)

Studies were performed in 11 untreated patients with Graves' disease who agreed to cooperate after explanation of the purpose of our study. The results on thyroid function tests are shown in Table 1.

Organ culture of thyroid slices

Thyroid tissue (8–10 mg) was obtained by Silverman needle biopsy. Tissue from each patient was divided into 5 slices and weighed, and slices were then cultured for 7 days in the organ culture system developed in our laboratory, details of which were previously reported (Hamada et al. 1983).

Several thyroid stimulators were tested in this organ culture system: 1) IgG (1.3 mg/ml) obtained from the same Graves' patient, 2) IgG obtained from other untreated patients, 3) normal IgG or 4) bovine TSH (Sigma 1.3 mIU/ml), was added to slices from the first day of culture. IgG were absorbed by protein A-Sepharose 4B columns, eluted with 0.1 M glycine buffer pH 2.5, dialysed with phosphate buffer pH 7.4 and concentrated to 10 mg/ml protein by an Amicon concentrator. In order to determine the concentrations of T3 in the culture media, media were changed daily, and the concentrations of T3 were measured by RIA and expressed as the concentration in the media divided by wet weight of thyroid slices.

The concentrations of thyroidal cAMP were measured by RIA on the last day of culture after incubation with 10 μmol theophylline at 37°C for 30 min.

Results

Fig. 1 shows the results of 11 patients. Values shown are the ratios of T3 in media with stimulators compared to T3 without stimulators. In order to eliminate the possibility of T3 release due to destruction by slicing and/or the culture procedure, T3 measurements were begun from the 2nd day of culture. When slices were cultured with TSH, the concentrations of T3 in media increased significantly to a ratio of 7.0 on the 5th day. When autologous IgG was added in the culture media, the concentrations of T3 increased to a ratio of 5.4 on the 5th day. The concentrations of T3 in media were not significantly affected when slices were cultured with IgG obtained from other untreated patients with Graves' disease.

The thyroidal cAMP concentrations increased

<p>| Table 1. Thyroid function in 11 untreated patients with Graves' disease. |
|-----------------------------|---------------|---------------|---------------|---------------|---------------|</p>
<table>
<thead>
<tr>
<th>Cases</th>
<th>T3 (ng/dl)</th>
<th>T4 (μg/dl)</th>
<th>FT4 (ng/dl)</th>
<th>TBIAb* (%)</th>
<th>HTACS** (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 A.Y.</td>
<td>16 F</td>
<td>803</td>
<td>34</td>
<td>4.1</td>
<td>44</td>
</tr>
<tr>
<td>2 T.T.</td>
<td>29 F</td>
<td>756</td>
<td>33</td>
<td>4.6</td>
<td>43</td>
</tr>
<tr>
<td>3 S.K.</td>
<td>49 F</td>
<td>594</td>
<td>18</td>
<td>3.7</td>
<td>66</td>
</tr>
<tr>
<td>4 M.M.</td>
<td>24 M</td>
<td>349</td>
<td>21</td>
<td>4.0</td>
<td>69</td>
</tr>
<tr>
<td>5 M.H.</td>
<td>35 F</td>
<td>406</td>
<td>17</td>
<td>3.9</td>
<td>29</td>
</tr>
<tr>
<td>6 E.S.</td>
<td>57 F</td>
<td>427</td>
<td>14</td>
<td>5.5</td>
<td>52</td>
</tr>
<tr>
<td>7 K.U.</td>
<td>34 F</td>
<td>439</td>
<td>13</td>
<td>3.3</td>
<td>28</td>
</tr>
<tr>
<td>8 N.T.</td>
<td>19 F</td>
<td>892</td>
<td>19</td>
<td>6.0</td>
<td>56</td>
</tr>
<tr>
<td>9 M.O.</td>
<td>32 F</td>
<td>767</td>
<td>22</td>
<td>4.9</td>
<td>43</td>
</tr>
<tr>
<td>10 T.N.</td>
<td>56 F</td>
<td>322</td>
<td>10</td>
<td>3.6</td>
<td>27</td>
</tr>
<tr>
<td>11 H.Y.</td>
<td>58 F</td>
<td>535</td>
<td>23</td>
<td>5.0</td>
<td>56</td>
</tr>
</tbody>
</table>

TBIAb* (TSH binding inhibiting antibody): positive > 15% modified HTACS** (human thyroid adenylate cyclase stimulator): positive > 150%. 

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Fig. 1.
Ratio of $T_3$ concentrations in media of Graves’ thyroid slices after incubation with or without stimulator (mean ± SEM, n = 11). * Significantly different from controls by one-tailed $t$-test ($P < 0.05$). ** Significantly different from controls by one-tailed $t$-test ($P < 0.01$)

significantly in response to both TSH and autologous IgG, but not to IgG from other untreated patients (Fig. 2). However, the increase of cAMP by autologous IgG was less than 2-fold.

As shown in Fig. 3, when IgG of 11 untreated patients with Graves’ disease were incubated with autologous thyroid slices, notable increase in $T_3$ in the media were invariably observed. However, when the same Graves’ IgG were incubated with allogeneic slices, no increase in medium $T_3$ was noted, with one exception, i.e., IgG from patient No. 3 stimulated thyroid slices from patient No. 6. Incubation with TSH resulted in increases in $T_3$ from all tissues tested.

Discussion

Our present study indicates that autologous IgG or TSH stimulated the release of $T_3$ as well as the production of cAMP, in thyroid slices obtained

Fig. 2.
The increase of cAMP production of untreated Graves’ thyroid slices in response to stimulators on the last day of culture.

<table>
<thead>
<tr>
<th>untreated Graves’ tissues</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<td></td>
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<tr>
<td>TSH</td>
<td>O</td>
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<td></td>
</tr>
</tbody>
</table>

Fig. 3.
Thyroid tissues from 11 untreated Graves’ patients are listed along the abscissa and stimulators (autologous Graves’ IgG and TSH) are listed along the ordinate. Circles represent a > 1.5-fold increase in medium $T_3$ concentrations than controls on the 5th day in response to stimulators and the negative indicates no increase in $T_3$. 

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from untreated patients with Graves' disease, whereas, IgG obtained from other untreated patients did not stimulate the release of T₃ nor the production of cAMP.

There are already numerous reports on the production of cAMP in normal thyroid slices during brief incubation with Graves' IgG (McKenzie et al. 1978; Zakarian & McKenzie 1978, 1980; Kuzuya et al. 1980; Adams 1981). Our previous results with an organ culture system using normal thyroid slices also showed increased production of thyroidal cAMP in response to Graves' IgG (Nagataki 1982; Hamada et al. 1983). The release of thyroid hormone from thyroids is stimulated by Graves' IgG in various conditions. The release of labelled hormones in response to Graves' IgG in mice is a well known phenomenon. It has been reported that Graves' IgG stimulates the release of hormone in vitro from mouse thyroid lobes (Adams & Purves 1956) and from slices of porcine thyroids (Laurberg & Weeke 1975; Kendall-Taylor & Atkinson 1980). However, the release of hormone from human thyroid slices was reported to be much more variable.

Our previous studies failed to demonstrate significant increases of T₃ release in organ culture system of normal human thyroid slices in spite of significant increases of thyroidal cAMP during 5–7 days of culture with Graves' IgG (Nagataki 1982; Hamada et al. 1983). These results indicate that in addition to the discrepancy between TSH-binding inhibiting and thyroidal cAMP stimulating activity, a discrepancy exists between thyroidal cAMP stimulating and thyroidal hormones releasing activity. Adams et al. (1974) reported that serum concentration of ¹³¹I increased in response to daily infusions of pooled Graves' sera in 5 normal subjects who had received ¹³¹I and had been given 75 μg T₃ daily. The discrepancy between their results and ours is not clear, however, they injected pooled sera obtained from many patients into normal subjects, while individual sera from Graves' patients were tested in vitro in the present study.

In contrast to the response in normal thyroid slices, autologous IgG stimulated both the production of cAMP and the release of T₃ in thyroid slices from untreated patients with Graves' disease, but neither the production of cAMP nor the release of T₃ was stimulated by IgG obtained from other untreated patients with Graves' disease. As reported previously (Kuzuya et al. 1980), cAMP production in response to allogeneic IgG was much lower in Graves' thyroids obtained at surgery after thionamide therapy than in normal thyroids. In the present study, thyroids obtained from untreated Graves' patients did not respond to allogeneic Graves' IgG. The response of thyroidal cAMP production to Graves' IgG is very different between Graves' and normal thyroids.

The results of the present experiments that Graves' IgG are highly specific for autologous thyroids in releasing T₃ have never been previously reported. Although there are a large body of evidences indicating that circulating Graves' IgG stimulates the production of thyroidal cAMP and the release of hormone from thyroids, these previous studies did not examine the possible specificity of Graves' IgG for autologous thyroids.

Further studies are required to explore this specificity, but our results strongly suggest that Graves' IgG are highly specific for autologous thyroids as far as T₃ secretion is concerned. The self-restriction observed in patients with Graves' disease in the present study is considered to present a new avenue for exploring the pathogenesis of Graves' disease.

Immunologically such a self-restrictive phenomenon could be explained by two types of self-recognition mechanisms (Hood et al. 1978; Adams 1981). One is the recognition of self major histocompatibility complex (MHC) products, and the other is the recognition of immunoglobulin idiotypes by specific anti-idiotype antibodies. Although anti-DR antibodies have been detected in other autoimmune diseases such as rheumatoid arthritis (Searles et al. 1983) and systemic lupus erythematosus (Okudaira et al. 1982), autoantibodies against self MHC products have not been found in autoimmune thyroid diseases. DR antigens, however, are present in cultured thyroid cells obtained from patients with Graves' disease (Hanafusa et al. 1983).

Recently two important papers have been reported in Lancet (Kendal-Taylor et al. 1984; Bottazzo et al. 1983). One is the evidence that thyroid-stimulating antibody (TSAb) is produced in the thyroid gland (Kendal-Taylor et al. 1984). They concluded that the increase in TSAb concentration in the thyroid vein indicates production of antibodies by lymphocytes within the thyroid and could explain why removal of the thyroid results in disappearance of TSAb from the circulation. We used the thyroid slices from untreated
Graves’ patients as materials, and thyroidal lymphocyte(s) may play an important role for stimulating thyroid hormone secretion synergistically with autologous IgG. The other paper (Bottazzo et al. 1983) presents a fascinating hypothesis based on the discovery of aberrant expression of HLA-DR antigen on thyocytes in Graves’ disease. They supposed that viral infections or other environmental factors, not yet confirmed, could induce release of interferon α or β. They would also activate T lymphocytes with the subsequent release of interferon γ, which would enhance expression of DR antigens on antigen presenting macrophages and endothelial cells, and also induce and maintain aberrant expression of DR in neighbouring epithelial cells especially in genetically predisposed individuals. We cannot prove the existence of anti-DR antibody in the peripheral circulation in Graves’ disease, but within the thyroid the possibility of the interaction between DR antigen and anti-DR antibody cannot be denied. From these standpoints, our methods using the thyroid slice from untreated Graves’ patients are ideal to observe the total effect of autologous IgG.

Regarding idiootype-anti-idiootype regulation, the presence of anti-thyrotropin anti-idiootype antibody could be speculated as being the mechanism of hyperthyroidism in the patients with Graves’ disease (Islam et al. 1983; Burman & Baker 1985). We must consider not only the heterogeneity of Graves’ IgG but also the abnormality of Graves’ thyroid including thyroidal lymphocytes and clarify the characteristics of those lymphocytes.

Susceptibility for Graves’ disease was reported earlier to be controlled by immunoregulatory genetic haplotypes of HLA and Gm (Nagataki 1982; Tamai et al. 1985). In our present study an exceptional IgG obtained from one of the patients stimulated untreated allogeneic thyroid slices. This patient may have had the same immunoregulatory genes of susceptibility as the donor patient of the thyroid slices which were used.

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References


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