Relationship between Potency of Blocking Type Thyrotropin-binding Inhibitor Immunoglobulin in Three Women with Primary Myxedema and Thyroid Function of Their Neonates

HIROAKI INOMATA, NOZOMU SASAKI, KIYOE TAMARU, HIDEO USHIKU, HIROO NIIMI AND HIRONORI NAKAJIMA

Department of Pediatrics, School of Medicine, Chiba University, Chiba 280

Abstract

Three neonates born to three mothers with primary myxedema who have thyrotropin-binding inhibitor immunoglobulin (TBII) were continually examined after birth. One neonate showed a high TSH level in mass-screening for congenital hypothyroidism and developed transient hypothyroidism. Her TBII disappeared at 114 days of age, and she remained euthyroid after discontinuation of thyroxin replacement at 146 days of age. The other two neonates were euthyroid, though they had positive TBII.

In three mothers, the doses of IgGs that inhibited 125I-TSH binding to the level of 50% were compared. The potency of IgG from the mother whose neonate developed hypothyroidism was stronger than that of IgG from the other two mothers. And the elevation of cAMP induced by bovine TSH in suspension culture with porcine thyroid follicles was significantly reduced in the presence of IgG from the three mothers when compared with normal IgG. The thyroid-stimulation blocking activity was more potent in the mother whose neonate developed hypothyroidism than in the other two mothers. This study suggests that the thyroid function of neonates born to primary myxedema with blocking type TBII is influenced by the potency of TSH-binding inhibitor and thyroid-stimulation blocking activity of the mother.

Recently, the presence of thyroid-stimulation blocking and thyrotropin-binding inhibitor immunoglobulin (blocking type TBII) in the serum of patients with primary myxedema was reported (Endo et al., 1978; Konishi et al., 1983). And neonatal transient hypothyroidism due to maternal blocking type TBII in the serum of patients with primary myxedema was reported (Endo et al., 1978; Konishi et al., 1983). And neonatal transient hypothyroidism due to maternal blocking type TBII has been reported (Matsuura et al., 1980; Iseki et al., 1983; Ninomiya et al., 1983; Takasu et al., 1984; Ishihara et al., 1985). More recently, Arikawa et al. (1985) described three neonates delivered from different three mothers with primary myxedema. All three mothers had blocking type TBII, but one neonate showed transient hypothyroidism due to TBII. However, the other two neonates were euthyroid.

Recently, we experienced three neonates who were delivered from three mothers with blocking type TBII. Thus, we have attempted to clarify the relationship between the neonatal thyroid functions and thyroid-
stimulation blocking (TSB) activity and TSH-binding inhibitor (TBI) activity presented in the serums of their mothers.

Materials and Methods

Serum T₄, T₃ and TSH were measured by RIA using commercially available kits. Antithyroglobulin (TGHA) and antithyroid microsomal antibodies (MCHA) were measured by a hemaggutination method using commercial kits (Fujizoki Co., Tokyo, Japan). Normal ranges had a dilution factor smaller than 1 to 100.

Crude IgG fractions were precipitated from serum with polyethylene glycol according to the method of Smith and Hall (1981). Purified IgG were prepared by DEAE-cellulose or DEAE-sephadex column chromatography.

TBII was determined by radioreceptor assay using a kit purchased from S. R. S. Limited (Cardiff, UK) (Shewring and Smith, 1982). Crude or purified IgG were used in TBII measurement.

Thyroid-stimulation blocking activity was determined as follows: suspension culture of thyroid follicles was carried out by a modification of the method of Nitsch et al. (1980). Fresh porcine thyroid glands were cut into small pieces and were suspended in Dulbecco's calcium- and magnesium-free salt solution (PBS (-)). Then to the fragments was added 0.1% collagenase and they were agitated for 30 min at 37°C. After centrifugation at 200 g for 10 min, thyroid follicles were obtained. The cells were placed in Ham's F 12 medium containing 0.5% calf serum, then cultured at 37°C for 2 days in a humidified atmosphere of 5% CO₂ in air. Purified test IgG and 1 mU/ml bovine TSH (Thytropar, Armour Pharmaceutical Co., Phoenix, AZ) were added in the suspension cultured thyroid follicles. After incubation for 2 h at 37°C in a humidified atmosphere of 5% CO₂ in air, the tubes were centrifuged and cAMP in the supernatant was measured in triplicate by RIA using a cAMP assay kit (Yamasa Shoyu Co., Chiba, Japan). The relative cAMP accumulation (%) was calculated as follows:

\[
\text{cAMP accumulated in the presence of test IgG} \times 100
\]

\[
\text{cAMP accumulated in the presence of normal IgG}
\]

Statistical analyses were based on Student's t-test.

Patient 1

This female infant was delivered by cesarean section because of cephalo-pelvic disproportion after 42 weeks of gestation and weighed 3750 g. At day 4 of life, the filter paper TSH level done for neonatal thyroid screening was revealed to be 259 µU/ml of whole blood. She had no symptoms or signs of hypothyroidism, and epiphysis of the distal femur was present and the size was normal at 42 days of age when she was hospitalized. Serum TSH, T₄ and T₃ levels were above 320 µU/ml, 3.9 µg/dl and 159 ng/dl, respectively. TGHA and MCHA were negative and TBII was positive at 87%. She was diagnosed as having hypothyroidism and 25 µg per day of L-T₄ was given at 43 days of age (Fig. 1). TSH and T₄ were immediately normalized by the therapy. The value of TBII gradually decreased and became negative at 114 days of age. L-T₄ therapy was discontinued at 146 days of age. She has remained euthyroid, and her physical and mental development are normal.

Patient 1-M (the mother of patient 1)

She was 32 years old at the birth of patient 1. At 30 years of age, facial puffiness, general malaise, hoarseness and irregular menstruation developed. She had no goiter. At that time, her serum TSH was above 320 µU/ml, T₄ was below 1.0 µg/dl and TGHA was negative. L-T₄ therapy was started and continued thereafter. When patient 1 was hospitalized, patient 1-M was treated with 100 µg L-T₄ per day and her serum TSH was slightly high, T₄ was low and TBII was very potent at 98%. After the L-T₄ dose was increased to 150 µg per day, serum TSH and T₄ became normal (Table 1).

Patient 2

This female infant was delivered normally after 38 weeks of gestation and weighed 3213 g. At 5 days of age, she received neonatal thyroid mass-screening, and her filter paper TSH was normal. She had no clinical symptoms and signs of hypothyroidism at 30 days of age, but her thyroid function was examined because her mother was affected with chronic thyroiditis. Her serum basal TSH was 2.4 µU/ml and rose to 24.3 µU/ml after TRH injection. Serum T₄ and T₃ were 9.9 µg/dl and 222 ng/dl, respectively. TGHA and MCHA were positive and TBII was weakly positive at 16.2%. At 64 days of age, TSH, T₄ and T₃ remained within the normal range and TBII became nega-
Fig. 1. Transient hypothyroidism due to maternal blocking type TBII (patient 1). Vertical columns show the normal range.

Table 1. Clinical data for three mothers with primary myxedema

<table>
<thead>
<tr>
<th></th>
<th>Age years</th>
<th>TSH (\mu U/ml)</th>
<th>T4 (\mu g/dl)</th>
<th>T3 (ng/dl)</th>
<th>TGHA</th>
<th>MCHA</th>
<th>TBII %</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1-M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at onset</td>
<td>30</td>
<td>320</td>
<td>1.0</td>
<td>ND</td>
<td>(&lt;10^2)</td>
<td>ND</td>
<td>ND</td>
<td>none</td>
</tr>
<tr>
<td>at this study</td>
<td>32</td>
<td>25.6</td>
<td>4.8</td>
<td>110</td>
<td>(&lt;10^2\times10^2)</td>
<td>98</td>
<td>LT4 100 (\mu g)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>1.0</td>
<td>9.4</td>
<td>139</td>
<td></td>
<td></td>
<td></td>
<td>LT4 150 (\mu g)</td>
</tr>
<tr>
<td>Patient 2-M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at onset</td>
<td>25</td>
<td>85.6</td>
<td>1.8</td>
<td>ND</td>
<td>(\times10^2\times40^2)</td>
<td>ND</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>at this study</td>
<td>31</td>
<td>3.1</td>
<td>6.2</td>
<td>152</td>
<td>(\times160^2\times320^2)</td>
<td>83</td>
<td>LT4 150 (\mu g)</td>
<td></td>
</tr>
<tr>
<td>Patient 3-M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at onset</td>
<td>26</td>
<td>230</td>
<td>1.0</td>
<td>30</td>
<td>(\times40^2\times160^2)</td>
<td>ND</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>at this study</td>
<td>32</td>
<td>18.3</td>
<td>6.0</td>
<td>137</td>
<td>(\times80^2\times320^2)</td>
<td>29</td>
<td>DT 120 mg</td>
<td></td>
</tr>
</tbody>
</table>

TGHA, Thyroglobin hemagglutinating antibodies; MCHA, Microsomal antigen hemagglutinating antibodies; ND, Not done; DT, Desiccated thyroid.
Table 2. Laboratory data for two euthyroid neonates whose mothers had blocking type TBII.

<table>
<thead>
<tr>
<th>Age</th>
<th>TSH µU/ml</th>
<th>T4 µg/dl</th>
<th>T3 ng/dl</th>
<th>TBII %</th>
<th>TGHA</th>
<th>MCHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 2</td>
<td>30 days</td>
<td>2.4</td>
<td>9.9</td>
<td>222</td>
<td>16.2 × 40^2</td>
<td>×80^2</td>
</tr>
<tr>
<td></td>
<td>64 days</td>
<td>24.3 (30 min, after TRH loading)</td>
<td>1.2</td>
<td>6.7</td>
<td>197</td>
<td>2.9</td>
</tr>
<tr>
<td>Patient 3</td>
<td>10 hrs</td>
<td>34.5</td>
<td>10.0</td>
<td>75</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>17 days</td>
<td>4.1</td>
<td>12.2</td>
<td>ND</td>
<td>33.6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>58 days</td>
<td>8.6</td>
<td>10.7</td>
<td>ND</td>
<td>2.9</td>
<td>ND</td>
</tr>
</tbody>
</table>

tive at 2.9% (Table 2).

Patient 2-M (the mother of patient 2)
She was 31 years old at the birth of patient 2. At 17 years of age, general malaise and generalized edema developed. At the age of 25 years, her serum TSH and T4 were 85.6 µU/ml and 1.8 µg/dl, respectively. TGHA was ×100 and MCHA was ×1600. Goiter was not noticed. She was diagnosed as having chronic thyroiditis and treated with L-T4. When patient 2 was seen, serum TSH, T4 and T3 of patient 2-M were normal with the administration of 150 µg L-T4. TGHA and MCHA were positive and TBII was strongly positive at 83% (Table 1).

Patient 3
This male infant was delivered normally after 38 weeks of gestation and weighed 2967 g. Because her mother had chronic thyroiditis, thyroid function was examined at 10 hours after birth and revealed to be normal (TSH, T4 and T3 were 34.5 µU/ml, 10.0 µg/dl and 75 ng/dl respectively). At 17 days of age, TSH, T4 and T3 were still normal, but, his serum TBII was positive at 33.6%. At 58 days of age, TBII became negative (Table 2).

Patient 3-M (the mother of patient 3)
She was 32 years old at the birth of patient 3. At 26 years old, general malaise, facial puffiness, hoarseness and constipation developed. Goiter was not noticed. Her serum TSH, T4 and T3 were 230 µU/ml, below 1.0 µg/dl and 30 ng/dl, respectively. TGHA was ×1600 and MCHA was ×25600. She was made a diagnosis of chronic thyroiditis and treated with desiccated thyroid. After she delivered patient 3, she was given 120 mg desiccated thyroid and her serum TSH, T4 and T3 were 18.3 µU/ml, 6.0 µg/dl and 137 ng/dl, respectively, and TBII was 29% (Table 1).

Results

Comparison of potency of TBII presented in serum of three mothers
IgGs of patient 1-M, 2-M and 3-M were diluted by normal IgG, and dilution curves of inhibition of ^125I-TSH binding to the receptor are shown in Figure 2. The doses of IgGs that inhibited ^125I-TSH binding to the level of 50% were 0.4, 0.7 and above 10 mg/ml in patient 1-M, 2-M and 3-M. Patient 1-M, whose neonate developed hypothyroidism, had the most potent TBII activity.

Comparison of TSB activity in three mothers
The effect of IgGs prepared from three mothers and normal subject on TSH stimulation of thyroid adenylate cyclase was examined. Table 3 shows the dose-response results covering the dose range 5–20 mg/ml obtained in the presence of 1 mU/ml TSH. At any concentration of IgG except at 10 mg/ml IgG of patient 3-M, the relative cAMP accumulations of three mothers were significantly lower than that of normal subjects. TSB activity of IgG prepared from patient 1-M was 2.6 and 15.7 times more potent than that of patient 2-M and of patient 3-M, respectively, at 10 mg/ml IgG concentration. And at 15 mg/ml IgG concentration, TSB activity of patient 1-M was
Table 3. Relative cAMP accumulations for three mothers

<table>
<thead>
<tr>
<th>Dose of IgG</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subject</td>
<td>100 ± 5.7</td>
<td>100 ± 22.8</td>
<td>100 ± 2.0</td>
<td>100 ± 17.4</td>
</tr>
<tr>
<td>Patient 1-M</td>
<td>6.4 ± 0.1*</td>
<td>6.7 ± 0.1**</td>
<td>4.9 ± 0.3*</td>
<td>10.8 ± 2.6*</td>
</tr>
<tr>
<td>Patient 2-M</td>
<td>50.0 ± 12.3**</td>
<td>17.5 ± 0.2**,</td>
<td>7.8 ± 1.1*,</td>
<td>12.8 ± 0.7*,</td>
</tr>
<tr>
<td>Patient 3-M</td>
<td>n. d.</td>
<td>105 ± 33.0***</td>
<td>35.0 ± 8.7*,</td>
<td>n. d.</td>
</tr>
</tbody>
</table>

Calculation of relative cAMP accumulation is explained in the text.

Data are shown as M ± S. D. (n=3). n. d.: not done.

*(p<0.001), **(p<0.005), *** (not significant) compared to normal subject.
a (p<0.001), b (p<0.005), c (p<0.025), d (not significant) compared to patient 1-M.

1.6 and 7.1 times more potent than that of patient 2-M and of patient 3-M, respectively.

Discussion

We described one patient with neonatal transient hypothyroidism due to transplacental blocking type TBII from her mother with primary myxedema. Previously similar cases were reported by Matsuura et al. (1980), Iseki et al. (1983), Ninomiya et al. (1983), Takasu et al. (1984), and Ishihara et al. (1985). The present family case is the sixth. The age of disappearance of the maternal TBII has been reported as three to ten months of age in previous reports. In this patient 1, TBII disappeared during 100 to 114 days of age. This patient was the first case detected in neonatal mass-screening for congenital hypothyroidism. In Japan, 98% of neonates have been screened, and 501 cases of congenital hypothyroidism and...
91 cases of transient hypothyroidism were detected by March 31, 1985 (Nakajima et al., 1985).

Furthermore, we presented two euthyroid neonates born to two different mothers with primary myxedema who had blocking type TBII. Arikawa et al. (1985) reported that IgG of a mother who delivered transient hypothyroid neonates had 10–60 times more potent TBII activity and a much stronger inhibitory effect on TSH-stimulated adenylate cyclase activity than those of two mothers who delivered euthyroid neonates. In our report, IgG of patient 1-M who delivered transient hypothyroid neonate had 1.75 and above 25 times more potent TBII activity than that of patient 2-M and patient 3-M, respectively. And TSB activity of patient 1-M's IgG was 1.6–2.6 times more potent than patient 2-M's IgG and 7.1–15.7 times more than patient 3-M's IgG. This result suggests that thyroid function of neonate born to primary myxedema with positive blocking type TBII is influenced by the potency of TBII and TSB activity of mother. Patient 3 had almost the same TBII activity as that of his mother, and yet his thyroid function was normal. One possibility is that TSB activity of patient 3 was less than that of his mother. His TSB activity could not be compared to that of his mother, because an insufficient blood sample was taken from patient 3. Another possibility is that the sensitivity of neonatal thyroid to TBII in vivo was different from that of mothers. Further studies are required for to clarify the heterogeneity of blocking type TBII.

Acknowledgements

We wish thank Drs. N. Momotani (Ito Hospital), K. Ito (Chiba University Hospital) and R. Kimura (Asahi Chuo Hospital) for showing us the clinical and laboratory data for each mother at her first visit.

References


