CASE REPORT

Potent thyrotrophin receptor-blocking antibodies: a cause of transient congenital hypothyroidism and delayed thyroid development

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Abstract

Objective: We describe an infant with surprisingly severe neonatal hypothyroidism due to transplacental passage of thyrotrophin receptor (TSH-R)-blocking antibodies (TBAb).

Design and methods: TBAb were detected using a cell line which stably expresses the human TSH-R and a cAMP-responsive luciferase reporter by their ability to inhibit TSH-stimulated luciferase expression. Potent TBAb were detected in maternal serum and initially in the infant’s serum but, in the latter, TBAb decreased over time to within the reference range by 3–4 months of age, illustrating the transient nature of this condition.

Results: The thyroid function of this child did not return to normal on withdrawal of thyroxine therapy at 16 months of age when he developed transient compensated hypothyroidism.

Conclusions: We propose that the presence of potent TBAb in utero and in the first weeks of life may have implications for the development of a normally sized thyroid gland. We have demonstrated the presence of TBAb in the mother’s milk and, as far as we are aware, this is the first such report. However, the TBAb in the milk probably did not contribute significantly to hypothyroidism in the child, given the reducing antibody titre in his circulation.

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Introduction

Autoantibodies to the thyrotrophin receptor (TSH-R) that block (TBAb) the effects of thyrotrophin (TSH) (1, 2) have been described in the serum of some patients with atrophic thyroiditis and Hashimoto’s thyroiditis. TBAb have a pathogenic role in the development of hypothyroidism. During pregnancy, passage of maternal TBAb across the placenta can cause transient hypothyroidism in the neonate (reviewed in 3). Transient congenital hypothyroidism (CH) due to TBAb generally occurs in infants of mothers with a history of autoimmune hypothyroidism who are taking thyroxine or have undiagnosed hypothyroidism, and accounts for 1–2% of cases of congenital hypothyroidism (4, 5). TBAb are found in both maternal and infant serum at birth but gradually clear from the infant’s circulation after 3–4 months. This is usually accompanied by resumption of normal thyroid function.

Materials and methods

Thyroid function tests

Serum concentrations of TSH, free thyroxine (T4) and free tri-iodothyronine (T3) were measured on an ADVIA Centaur automated immunoassay analyser (Bayer plc, Newbury, Bucks, UK). Thyroid peroxidase antibodies were measured by competitive immunoassay on a Roche Diagnostics Elecsys 2010 automated immunoassay analyser (Roche Diagnostics GmbH, Mannheim, Germany). Thyroglobulin was measured using the Pasteur thyroglobulin immunoradiometric assay (Bio-Rad, Marnes La Coquette, France).

TBAb and TSH-R-stimulating antibodies

Serum concentrations of TBAb and TSH-R-stimulating antibodies (TSAb) were measured using the previously described lulu* cell line (Chinese hamster ovary cells stably transfected with recombinant human TSH-R and

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a cAMP-dependent luciferase reporter) (6). Lulu* were seeded at approximately 2 \times 10^3 cells per well in 96-well plates in Ham’s F12 containing 10% charcoal stripped calf serum. Thirty-six hours later cells were switched to assay buffer (see below). To measure TBAb, serum (10%) was incubated with lulu* in the presence of bovine TSH (1 mU/ml) in Ham’s F12 without 10% fetal calf serum (FCS). To measure TSAb, serum (10%) was incubated with lulu* in Hank’s balanced salt solution without sodium chloride (0.185 g/l CaCl₂, 0.4 g/l KCl, 0.06 g/l KH₂PO₄, 0.1 g/l MgCl₂, 0.1 g/l MgSO₄, 0.35 g/l NaHCO₃, 0.48 g/l Na₂HPO₄) containing 1.0 g/l n-glucose, 20 mM HEPES, 1.5% bovine serum albumin, 280 mM sucrose and 5% polyethylene glycol). All incubations were carried out for 5 h at 37 °C in 5% CO₂ in air. Where dilutions of sera were assayed, the total serum concentration was adjusted to a total of 10% by the addition of pooled euthyroid serum.

In each case, cAMP-dependent luciferase production was determined by measuring light in the presence of luciferin using a luciferase reporter assay (Promega UK Ltd, Southampton, UK) on a Perkin Elmer Applied Biosystems Tropix TR717 microplate luminometer (Perkin Elmer, Norwalk, CT, USA). Experiments were performed in duplicate or triplicate and the results expressed as an average. TBAb activity was expressed as an inhibition index (InI), calculated as follows: InI = \frac{\text{light (patient sample + euthyroid pool)}}{\text{light (euthyroid pool + TSH)}}. InI > 23 is considered positive (6). TSAb activity was expressed as a stimulation index (SI): the ratio of light output from the patient sample to light output from the euthyroid pool. SI > 1.5 is considered positive.

TBAb activity in milk was assessed by incubation of varying amounts of patient or control unpasteurised bovine milk with lulu* in the presence of bovine TSH (1 mU/ml) and Ham’s F12 without 10% FCS. Luciferase activity was measured as previously described.

All investigations were performed with the informed consent of the mother.

Case report

A 4.57 kg male infant, the first child of a 31-year-old mother, was born at 41 weeks of gestation after an uneventful pregnancy. Neonatal screening revealed a blood spot TSH of 330 mU/l (normal <10) and serum taken at 13 days of age confirmed primary hypothyroidism with a low free T4 (2.7 pmol/l) and an grossly increased TSH concentration (752 mU/l). He was jaundiced (bilirubin 381 µmol/l) and had coarse facial features. X-ray of his knee showed neither the distal femoral nor proximal tibial epiphyses present, consistent with hypothyroidism in utero. A serum thyroglobulin concentration of 215 µg/l confirmed the presence of thyroid tissue. A normally placed, small thyroid gland was found on ultrasound of the neck: left lobe length 0.7 cm, breadth 0.4 cm, right lobe length 0.7 cm, breadth 0.6 cm (normative data for term infants (7): lobe length (mean ± S.D.) range 1.94 (0.24) 0.9–2.5 cm and breadth 0.86 (0.16) 0.5–1.4 cm). No uptake of technetium-99 was observed. Thyroxine therapy was started on day 13 of life at a dose of 50 µg/day.

The infant’s mother had a 14-year history of atrophic hypothyroidism for which she was on thyroxine replacement therapy (free T4 24.9 pmol/l, free T3 5.0 pmol/l and TSH 0.5 mU/l at the time of the infant’s birth). Thyroid peroxidase antibodies were marginally increased (38.9 kU/l (reference range <32 kU/l)). She was clinically well, with no goitre, no evidence of thyroid eye disease or pretibial myxoedema. The maternal history of atrophic hypothyroidism and the infant’s negative radioisotope uptake scan raised the possibility of transient neonatal hypothyroidism due to TBAb and measurement of TBAb was therefore initiated.

Detection of TBAb in serum

TBAb activity (InI range 70–80%) was detected in maternal serum throughout the investigation (Fig. 1). TBAb (InI 76%) were also detected in the infant’s serum soon after birth, but decreased to within the reference range by 4 months of age and were not found in the infant’s serum thereafter (Fig. 1).

Since serum TBAb and TSAb may coexist (8) multiple dilutions of maternal serum were assayed for both TBAb and TSAb. Measurement of TSAb was performed in NaCl-free buffer since detection of TSAb is improved in the absence of salt. In contrast, TBAb activity was determined in culture medium containing physiological NaCl concentrations in which TSH is more effective at stimulating luciferase expression (6). Potent TBAb activity was confirmed in the maternal serum (Fig. 2) and was still detectable at a 100-fold dilution (0.1% maternal serum in the bioassay equivalent to 15 µg/ml IgG). TSAb activity was not detected at any dilution of the maternal serum.

![Figure 1 Measurement of TBAb in maternal and infant serum after birth. TBAb activity was assessed by luminescent bioassay. Serum (10%) was challenged against 1 mU/ml bovine TSH. InI ± S.E.M. was calculated for duplicate experiments (n = 3); maternal serum (open bars) and infant serum (hatched bars). Euthyroid patients (n = 31) had InI < 23% (shown by dotted line).](image-url)
Detection of TBAb in milk

TSAb have been described in the milk of mothers with Graves’ disease (9). We detected TBAb activity in milk expressed by the mother (Fig. 3). A dose-dependent inhibition of TSH stimulation was observed with increasing amounts of breast milk assayed in contrast to control milk, in which TSH stimulation was not significantly different.

Withdrawal of thyroxine

Thyroxine therapy was required at birth, but the growing infant did not require an increase in dose to maintain TSH within the reference range and subsequent reductions in the dose of thyroxine were well tolerated. However, complete withdrawal of thyroxine at 16 months resulted in a transient compensated hypothyroidism (Table 1). At this time, he had a normal response to a perchlorate discharge test and remained clinically well. TBAb block the action of TSH and the uptake of radioactive isotope by the thyroid (references in 3) and account for lack of technetium-99 uptake in the earlier scan. By 2 years of age, his thyroid function had fully recovered and TSH levels returned to within the reference range.

Discussion

We have described an infant with profound hypothyroidism at birth and in utero due to transplacental passage of TBAb. Potent TBAb were detected in maternal serum and in the infant’s serum at birth using a luciferase-based bioassay. TBAb activity in the infant’s serum decreased to within the reference range by 3–4 months of age, illustrating the transient nature of CH due to maternal TBAb. The thyroid function of this child did not return to normal on withdrawal of thyroxine therapy when he developed a transient compensated hypothyroidism. We speculate that this slow recovery of normal thyroid function was caused by the impairment of the normal growth and development of the thyroid gland by the TBAb present before and after birth. Most cases of hypothyroidism due to TBAb are transient, but there has been one previous report of an infant with permanent thyroid damage due to TBAb (10). Hence it is important to monitor the response to thyroxine withdrawal in these infants carefully.

Transplacental passage of TBAb resulted in delayed development of this infant’s thyroid gland, which was found to be small by ultrasound scan shortly after birth.

Table 1 Biochemical investigations in the neonate.

<table>
<thead>
<tr>
<th>Age of infant</th>
<th>TSH (mU/l)</th>
<th>Free T4 (pmol/l)</th>
<th>Free T3 (pmol/l)</th>
<th>Comments</th>
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<tbody>
<tr>
<td>13 days</td>
<td>752</td>
<td>2.7</td>
<td>9.0</td>
<td>Thyroxine commenced (50 μg)</td>
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<tr>
<td>26 days</td>
<td>1.1</td>
<td>35.3</td>
<td>6.3</td>
<td>Thyroxine dose reduced to 40 μg</td>
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<td>1 month</td>
<td>0.59</td>
<td>19.1</td>
<td>6.1</td>
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<td>17.4</td>
<td>6.1</td>
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<td>0.2</td>
<td>16.8</td>
<td>6.6</td>
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<td>26 months</td>
<td>3.5</td>
<td>14.4</td>
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Figure 2 TBAb activity determined by luminescent bioassay for various dilutions of maternal serum. Serum (made up to a total of 10% with euthyroid serum) was challenged against 1mU/ml bovine TSH. InlI calculated for a duplicate experiment are shown. Euthyroid patients had InlI <23% (shown by dotted line).

Figure 3 TBAb activity in milk. The graph shows the inhibition of stimulation by 1 μU/ml bovine TSH, due to varying concentrations of breast milk (shaded bars) and non-pasteurised bovine milk (open bars). Mean relative light units (RLU) for a duplicate experiment are shown.

Table 2 Detection of TBAb in milk.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Inhibition Index (nI)</th>
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<td>1</td>
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<td>1:10</td>
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<tr>
<td>1:250</td>
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Figure 3 TBAb activity in milk. The graph shows the inhibition of stimulation by 1 μU/ml bovine TSH, due to varying concentrations of breast milk (shaded bars) and non-pasteurised bovine milk (open bars). Mean relative light units (RLU) for a duplicate experiment are shown.
birth. Hypoplastic thyroid glands have also been described in some patients with TSH-R loss-of-function mutations (references in 11). Therefore it seems that TSH/TSH-R signalling is of importance for the development of a normally sized thyroid gland in humans in utero, although it has recently been demonstrated that TSH or a functional TSH-R is not required for the development of a normal sized thyroid gland in mice in utero (11).

Although functional TSH/TSH-R signalling seems to be required for production of a normal sized gland, the gland of the infant described here and those of previously described patients with inactivating mutations of the TSH receptor are normally sited and serum thyroglobulin is increased. These observations support the hypothesis that migration and development of the thyroid gland is TSH independent, although functional TSH-R signalling may be required to achieve a fully differentiated thyroid phenotype (12).

We have presented evidence for TBAb activity in breast milk. Others (9) have previously observed the presence of TBAb in breast milk but the clinical significance is not clear. We speculate that the TBAb in the milk did not contribute significantly to hypothyroidism in the child, since clearing of the antibodies from his circulation was evident over time.

This case has demonstrated that transplacental passage of TBAb can result in profound neonatal hypothyroidism at birth. It is essential to start thyroxine replacement without delay. The transient nature of the hypothyroidism can then be determined by measurement ideally of TBAb or indirectly through measurement of TSH-binding inhibitory immunoglobulins in maternal serum. Affected infants require thyroxine therapy until TBAb clears from their circulation, so allowing normal thyroid function to resume. However, potent TBAb in the infant’s circulation may damage or delay development of the thyroid gland, therefore thyroid function should be carefully monitored when thyroxine is withdrawn.

Acknowledgements

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References

3 McKenzie J & Zakarija M. Fetal and neonatal hyperthyroidism and hypothyroidism due to maternal TSH receptor antibodies. Thyroid 1992 2 135–159.

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