Thyroid Antigen-Antibody Nephritis: Possible Involvement of Fucosyl-GM1 as the Antigen

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Abstract

Hyperthyroidism, microscopic hematuria, and proteinuria developed in an 11-year-old girl. Proteinuria decreased during treatment of hyperthyroidism with an antithyroid drug. On admission, serum anti-thyroglobulin antibody, antimicrosomal antibody, and immune complex were present. The thyrotropin binding inhibitory immunoglobulin (TBII) level was low. On the other hand, an antibody to the ganglioside component (fucosyl-GM1) was detected by an enzyme linked immunosolvent assay (ELISA).

A thyroid biopsy specimen showed massive lymphocytic infiltration and interstitial fibrosis. A renal biopsy specimen showed marked proliferation of mesangial cells and increased mesangial matrix with focal segmental capillary wall abnormality. Electron microscope studies demonstrated mild paramesangial dense deposits.

By indirect immunofluorescence, granular glomerular basement membrane and mesangial staining were not detected with rabbit antibody to thyroglobulin, but were detected with rabbit antibody to fucosyl GM1. Fucosyl GM1 was also seen along the basilar aspect of the thyroid follicular epithelial cells.

These observation suggests the development of glomerulonephritis mediated by thyroid antigen, particularly ganglioside component.

Many forms of human immune complexes mediate glomerulonephritis, but, there has been only limited success in identifying the specific antigens causing the disease. To

date bacterial, viral, thyroïdal, and nuclear antigens have been characterized (1). Recently, O'Regan *et al.* reported the accumulation of thyroglobulin (Tg) and thyroid microsomal antigen on the glomerular basement membrane in the kidney of a patient with hyperthyroidism and nephrotic syndrome, and suggested that these antigens caused immune complex-mediated nephritis (2).

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Patients suffering from Graves' disease and Hashimoto's thyroiditis are known to produce autoantibodies to the thyrotropin receptors that stimulate or inhibit the secretion of the thyroid hormone and generate the typical feature of the disease (3). The thyrotropin receptor is considered to contain both a glycoprotein and a ganglioside, which form a multireceptor complexes with high and low affinity binding capacities. Kohn *et al.* reported that the stimulating antibody exhibits species-specific binding with human thyroidal ganglioside (4) (5).

On the other hand, Sawada *et al.* reported the presence of anti-asialo GM1 and anti-fucosyl GM1 antibodies in sera from patients with Graves' and Hashimoto's disease, suggesting that asialo GM1 and fucosyl GM1 are components of the thyrotropin receptor (6) (7). To clarify the possible involvement of glycolipids in the immune complex-mediated nephritis as well as in the thyrotropin receptor, we examined the glycolipid antigen in the kidney and thyroid of a patient with hyperthyroidism

and proteinuria by an indirect immunofluorescence technique using anti-glycolipid antibodies.

Case Report

An 11-year-old girl was hospitalized for evaluation of palpitation and edema. She had had palpitation for 3 years prior to admission. On physical examination, her blood pressure was 177/110 mmHg. She weighed 41.5 kg and was 153 cm tall. Moderate pitting edema of the lower extremities was observed. The thyroid was enlarged and slightly tender.

Laboratory results were as follows: Hb 10 g/dl, serum BUN 78.3 mg/dl, creatinine 1.15 mg/dl, uric acid 12.1 mg/dl, and cholesterol, Na, K and Cl levels were within normal limits. Urinalysis showed many RBC and 5 to 10 WBC per field with 4(+) protein. Twenty-four-hour urinary protein excretion was 15 g/day. Anti-Tg antibody, antimicrosomal antibody, and immune complex were positive in the sera.

Fig. 1. Clinical course; Hyperthyroidism, microscopic hematuria, and proteinuria developed. Proteinuria decreased during treatment of hyperthyroidism with anti-thyroid drug.

The C₃, C₄, and CH50 levels were decreased.

Endocrinological studies showed a T₄ levels of 21.5 µg/dl, T₃ of 3.06 ng/ml, free-T₄ of 4.45 ng/dl, and a 24-hr 131I thyroid uptake of 67%. Thyroid scanning revealed an enlarged thyroid.

After one week, she lost consciousness and had hemiconvulsions, EEG and CT scanning at that time did not reveal any abnormal signs. During the next three weeks, renal function and her physical stigmata improved (Fig. 1). Two months after admission, a percutaneous renal biopsy and thyroid biopsy were performed.

Methods

1. Anti-TSH receptor antibody (TRAb)

The thyrotropin binding inhibitory immunoglobulin (TBII) was assayed by Smith's method (18). The anti-fucosyl GM1 antibody titer was determined by the enzyme linked immunosorbent assay (ELISA) (12). Briefly, 5 µg of fucosyl GM1 in 50 µg of ethanol was placed in each well of a polystyrene microtiter plate (Dynatec Lab, Inc.), and evaporated to dryness. After blocking each well with BSA-containing veronal buffer, the antiserum diluted serially was incubated at 37°C for 30 min followed with O-phenylenediamine and H₂O₂ at 30°C for 1 hr. The reaction was terminated by adding 20 µg of 8N H₂SO₄ and the enzyme activity was measured at 490 nm.

2. Tissue studies

The renal biopsy and thyroid biopsy materials

were cut into small pieces for biochemical examination and the portions were fixed in neutral-buffered formalin for light microscopy and in 2% glutaraldehyde for transmission electron microscopy. Snap-frozen sections were stained by an indirect immunofluorescence method with rabbit antisera to human thyroglobulin, asialo GM1 and fucosyl GM1 as the first antibody, and FITC-labeled goat anti-rabbit IgG antisera as the second antibody. Histochemical examination by direct immunofluorescence and the PAP technique to human IgG, IGA, IgM, C₃ and fibrin was also performed.

Results

Fucosyl GM1 antibody was detected in the sera of this patient by the ELISA method (Table). Fucosyl GM1 antibody was also found in other untreated cases of hyperthyroidism, and the antibody titer was decreased by therapy with antithyroid drug. In this case, the TBII level was low on admission but rose during the acute phase.

Light microscopy (Fig. 2): Nine glomeruli were present, and the most striking change was severe diffuse proliferation of the glomerular mesangial cells and matrices as disclosed by the hematoxylin-eosin and PAM stainings. The glomerular basement membrane was irregularly thickened. Moderate lymphocytic infiltration was seen in the interstitial tissue. The Pathological diagnosis was diffuse mesangial proliferative glomerulonephritis with focal segmental capillary

Table. Summary of serological data

	(normal range)	On admission	two weeks	two months
anti-Tg AB*1	(<100)	6400	1.2800	2500
anti-Microsome Ab*1	(<100)	40.9600	10.240	10.2400
anti-Fuc GM1 AB ²	(0.178±0.026SD)	0.347	0.345	0.239
TBII*3	(<15%)	6.5	31.4	34.6
C ₃	(50-120 mg/dl)	29	53	107
C ₄	(20-50 mg/dl)	12.7	19	34.4
immune complex*4	(<3.0)	40	10	<3.0

*1. hemoagglutination test *2. ELISA (optical density)

*3. radioreceptor assay by smith's methd. *4. Clq assay

wall abnormality. In the thyroid tissue, massive interstitial lymphocytic and plasmatic infiltration was found. Thyroid follicles were small and displayed marked marginal vacuolization.

Electron microscopy (Fig. 3): An ultrastructural study of the glomeruli confirmed the findings noted in light microscopy. Mild paramesangial and subendothelial dense deposits were seen, and segmental mesangial

Fig. 2. Light microscopy : (A) diffuse mesangial proliferative glomerulonephritis with segmental capillary wall abnormality (PAM). (B) interstitial lymphocytic infiltration and marked marginal vacuolization in thyroid follicle (H. E.)

Fig. 3. Electron microscopy : paramesangial dense deposits (▼) and segmental reticulosis of the lamina densa.

Fig. 4. (A) Renal biopsy specimen showing granular deposition of fucosyl GM1 mainly in the mesangium, (B) thyroid biopsy specimen demonstrating granular deposition of fucosyl GM1 along the basilar aspect of the follicular epithelial cell.

interposition was shown.

Immunofluorescent microscopy (Fig. 4): In the glomeruli, there were granular depositions of IgG, IgA, IgM and C₃ along the capillary walls and in the mesangium, but thyroglobulin was not detected. Indirect fluorescence studies showed granular deposition of fucosyl GM1 mainly in the mesangium. Sections taken from other patients with various glomerulopathies showed no fluorescence for fucosyl GM1. In the tissue of the thyroid, granular depositions of IgG, IgA, and fucosyl GM1 were seen along the basilar aspect of the follicular epithelial cells, but normal thyroid was not stained by anti-fucosyl GM1 antibody.

Discussion

The association in our patient of hyperthyroidism and proteinuria due to chronic glomerulonephritis suggests that these two entities may be linked by a thyroid antigen-antibody immune complex (2) (8) (9) (10). Weigel and Nakamura showed that a rabbit given periodic injection of arsanil-sulphanil thyroglobulin developed circulating antibodies to thyroglobulin which caused

thyroiditis and glomerulonephritis (1). Recently, O'Regan, *et al.* reported a patient with hyperthyroidism (2), who showed an elevated level of an antithyroglobulin antibody and an accumulation of thyroglobulin on the glomerular basement membrane. In our patient, circulating antithyroid antibodies were also detected, but human thyroglobulin was not demonstrated on the glomerulus of the patient by indirect immunofluorescence with anti-human thyroglobulin antiserum. On the other hand, a new antibody to fucosyl GM1 was detected by ELISA in the serum, and predominant staining with IgG and fucosyl GM1 was demonstrated on the glomerulus and thyroid follicular epithelial cells. Granular deposition of fucosyl GM1 on the thyroid follicular cell was mainly due to circulating immune complex, but in situ immune complex formation was not denied. In normal thyroid, fucosyl GM1 was not stained. Sawada reported that in thyroid carcinoma fucosyl GM1 was detected. Probably, in hyperthyroidism induction of fucosyl GM1 was accentuated.

Patients with chronic thyroiditis and Graves' disease often produce antibodies that stimulate secretion of the thyroid hormone. These autoantibodies bind to

thyrotropin receptors of thyroid follicular cells and compete with thyrotropin in the receptor binding. Other autoantibodies with the ability to bind the thyrotropin receptor inhibit the thyroid function. Such a variety of serum autoantibodies in thyroid diseases indicates the complexity of the thyrotropin receptor component (11). The thyrotropin receptor is considered to contain both a glycoprotein and a ganglioside component (5). Khon *et al.* showed that the initial recognition of thyrotropin requires the glycoprotein unit because one of the monoclonal antibodies has a blocking capacity (4). The other monoclonal antibodies with a stimulatory effect on the thyroid interacted with ganglioside. These findings suggested that a ganglioside or ganglioside-like structure is involved in a component of the thyrotropin receptor. In fact, GD1b and GT1b were found to be potent inhibitors of the binding of TSH using ^{131}I -labeled TSH. Also, Mullin *et al.* reported that a tissue-specific ganglioside of bovine thyroid inhibited TSH binding more strongly than GD1b and the molecule migrated close to fucosyl GM1 on thin layer chromatography (14). Fucosyl GM1 was found to be distributed in follicular epithelial cells by an immunohistochemical technique using the anti-fucosyl GM1 antiserum (Sawada K, unpublished data). Recently, Svennerholm reported that the human thyroid gland contained a much larger fraction of GM1, fucosyl GM1, and GD1d than bovine thyroid gland (19). Sawada *et al.* detected anti-asialo GM1 antibodies in the sera of Graves' and Hashimoto's disease by the complement fixation technique (6). Of twelve patients with Graves' disease, eleven showed a positive reaction, and the serum from patients with Hashimoto's thyroiditis was also positive. In Graves' disease, antibodies to fucosyl GM1 were also detected in the serum (7). Although there is evidence for the specific interaction of gangliosides with TSH, the gangliosides from the human

thyroid have not been fully characterized.

How anti-receptor antibodies are produced is not fully understood, but anti-idiotypic antibodies to antiviral or bacterial antibodies are felt to stimulate clones to produce autoantibodies, since many exogenous immunogens can trigger the idiotype-anti-idiotypic cascade (16). Another relevant finding is the presence of Yersinia enterocolitica (17) and cholera toxin that behave like thyrotropin. These antigen-antibodies form circulating immune complexes, and the formation of the immune complex is related to the onset of hyperthyroidisms and proteinuria. Improving these by using an anti-thyroid drug in our case suggests that the thyroid tissue might be supplying the fucosyl GM1 in the glomeruli during the active phase, but renal biopsy was performed in a later phase. Deposition of the glomerus was mild at that time.

The use of these antigens can provide new insight not only into the structure of the human TSH receptor, but also into the role of autoantibodies in Graves' disease. To examine the possible involvement of specific gangliosides in the function of the thyroid, recently developed methods such as direct binding to gangliosides separated on thin layer chromatographic plates should be performed.

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