Anterior Pituitary Cell Antibodies Detected in Hashimoto's Thyroiditis and Graves' disease

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Abstract

An immunofluorescence study using unfixed cryostat sections of rat pituitary glands was carried out on sera from 34 patients with Hashimoto's thyroiditis, 28 patients with Graves' disease, 10 patients with thyroid adenoma and 50 healthy subjects. After absorption of sera with rat liver tissues, 19 of 34 patients retained reactivity to anterior pituitary cell antibodies (PCA, 55.8%). On the other hand, immunofluorescence in anterior pituitary cells was faint and detected in only 2 of 28 patients with Graves' disease (7.1%) after absorption of their sera with rat liver acetone powder. A similar result was also obtained when PCA were compared in the sera of Hashimoto's thyroiditis and Graves' disease with high titers of thyroid microsomal auto-antibodies. PCA were detected neither in the sera of patients with thyroid adenoma nor in the healthy subjects.

The present study suggests that PCA were considerably more prevalent in Hashimoto's thyroiditis than in Graves' disease.

Pituitary cell antibodies (PCA) were frequently observed with isolated ACTH deficiency and insulin-dependent diabetes mellitus by immunofluorescence assay using cryostat sections of human pituitary gland (Mirakian *et al.*, 1982; Populard, 1982). This suggests that an autoimmune process may be implicated as one of the pathogenic factors in these disorders. On the other

hand, evidence is accumulating to suggest that both Hashimoto's thyroiditis and Graves' disease may be a representative disease associated with an autoimmune basis (Calder and Irvine, 1975; Volpe *et al.*, 1974; Volpe, 1977; Okita *et al.*, 1981). However, little attention has been paid so far to the prevalence of PCA in Hashimoto's thyroiditis and Graves' disease.

The aim of the present study was to determine the prevalence of PCA in patients with Hashimoto's thyroiditis as compared to Graves' disease.

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Materials and Methods

Sera

Sera were collected from 34 patients with Hashimoto's thyroiditis, 28 patients with Graves' disease and 10 patients with nontoxic thyroid adenoma. Fifty healthy subjects served as controls. Aliquots of the sera were absorbed with liver acetone powder (100 mg/ml serum) at 4°C for 24 hr.

PCA assay by biotin-avidin system

PCA were assayed by the method of Sugiura *et al.*, (1986). The anterior pituitary glands were obtained from Wistar male rats. Unfixed 5 μ m cryostat sections were used for the study. Aliquots of serum (25 μ l), after absorption with rat liver acetone powder, were added to sections on slides and kept for 30 min at room temperature in a humidified chamber. They were washed twice with PBS (pH 7.4). Twenty-five μ l biotinylated anti-human IgG solution (0.5 mg/ml PBS, Vector Lab. Inc., Calif.) was then added to the sections and slides were kept for 30 min at room temperature. After washing twice with PBS (pH 8.0), 25 μ l of FITC-labeled avidin solution (0.167 mg/ml PBS, Vector Lab., Inc.) was added to the sections and incubated at room temperature for an additional 30 min. After three washings with PBS, the sections were examined under an incident fluorescence microscope (Olympus BH-2).

Thyroid autoantibody assays

Thyroid microsomal autoantibodies (MHA) were assayed by passive microhaemagglutinin (Fujizoki, Tokyo). Antithyroglobulin antibodies also were measured with a commercially available kit (Fujizoki, Tokyo).

Diagnosis of thyroid diseases

Diagnosis of Hashimoto's thyroiditis and Graves' disease was based on the clinical history, the presence of diffuse goiter, thyroid autoantibodies, and serum T₄, T₃ and TSH concentrations. The patients with Graves' disease were untreated at the time of the study. Thyrotropin-binding inhibitor immunoglobulins (TBII) were assayed with a commercially available kit (Smith and Hall, 1974). All of the patients with Hashimoto's thyroiditis had been receiving L-T₄ (100 μ g/day) orally for periods varying from 6 months to 60 months. None of the patients had definite hypothyroidism prior to treatment. Diagnosis of thyroid adenoma was made from the findings including scintiscan, aspiration cytology and ultrasound studies on thyroid. Serum T₄ was measured with a RIA kit (Daiichi Radioisotope Labo., Tokyo). Serum T₃ and TSH were determined as previously (Kobayashi *et al.*, 1980). Statistical evaluation was made by X²-test.

Results

Sera from the patients with Hashimoto's thyroiditis, Graves' disease, thyroid adenoma and healthy subjects were tested for antibodies to anterior pituitary cells by this biotin-avidin system employing rat pituitary cryostat sections. The results are summarized in Table 1. After absorption of sera with rat liver tissues, 19 of 34 patients with Hashimoto's thyroiditis retained reactivity to anterior pituitary cells (55.8%). On the other hand, immunofluorescence was faint and detected in only 2 of 28 patients with Graves' disease (7.1%) after absorption of

Table 1. Prevalence of pituitary cell antibodies (PCA) in patients with thyroid diseases and healthy subjects

Group	No. of patients	No. of positive PCA	
		MHA < 25600 $\times$	MHA $\geq$ 25600 $\times$
Hashimoto's thyroiditis	34	1 (2.9)	18 (52.9) ^{#*}
Graves' disease	28	0 (0)	2 (7.1)
Thyroid adenoma	10	0 (0)	0 (0)
Healthy subjects	50	0 (0)	0 (0)

[#] The results after absorption of sera with rat liver acetone powder are shown.

* $p < 0.05$ vs. Graves' disease.

sera with rat liver acetone powder. In contrast, 10 sera from thyroid adenoma patients and 50 healthy subjects were all negative on PCA assay. PCA were tentatively compared in the sera of Hashimoto's thyroiditis and Graves' disease with relatively high titers of MHA ($\text{MHA} \geq 25600 \times$). The prevalence of PCA was significantly higher in the sera of the patients with Hashimoto's thyroiditis than that of Graves' disease ($p < 0.05$).

Discussion

The autoimmune nature of destructive hypophysitis has been suspected since 1962 (Goudies and Pinkerton, 1962), but the evidence of PCA had been scarce. Pouplard (1982) reported that 2 of 19 patients with panhypopituitarism were positive for PCA on rat or guinea pig. Many investigators reported that isolated ACTH deficiency was associated with Hashimoto's thyroiditis (Goudie and Pinkerton, 1962; Hume and Robert, 1967; Richtsmeier *et al.*, 1980). Kojima *et al.*, (1982) described a case of isolated ACTH deficiency associated with polyglandular failure.

In the present study, we have assayed PCA in the three thyroid diseases, determined by fluorescence with unfixed cryostat sections of rat pituitary gland. After absorption of sera with rat liver acetone powder, the prevalence of PCA was 55.8% in the sera of patients with Hashimoto's thyroiditis. In contrast, immunofluorescence in the sera of patients with Graves' disease was faint and almost disappeared following the addition of rat liver tissues. A similar result was obtained when PCA-positive patients with relatively high titers of MHA from the two autoimmune thyroid diseases were compared (Table 1). It is possible that PCA may often be associated with thyroid autoantibodies. The effect of the absorption of patient sera with human thy-

roid acetone powder was also examined. Immunofluorescence in anterior pituitary cells was still observed (data not shown). These results suggest that PCA were considerably more prevalent in Hashimoto's thyroiditis than in Graves' disease.

The present investigation does not discriminate between the cell type abnormalities of the pituitary gland. Nevertheless, our data lend support to the concept that sometimes Hashimoto's thyroiditis may extend to endocrine organ specific involvement when sera contain the organ specific antibodies (Volpé *et al.*, 1974; Volpé, 1977). Further studies are needed to see if there are some abnormalities in the hypothalamo-pituitary axis in PCA-positive patients, particularly those with Hashimoto's thyroiditis.

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