Anti-Pituitary Antibodies in Patients with Hypopituitarism and Their Families: Longitudinal Observation

KAZUO KAJITA, KEIGO YASUDA*, NORIYOSHI YAMAKITA*, TOSHIHIRO MURAI*, MASAFUMI MATSUDA*, HIROYUKI MORITA*, AKIHIRO MORI*, MASANORI MURAYAMA*, SHINOBU TANAHASHI**, MASAHIKO SUGIURA***, AND KIYOSHI MIURA*

Nagahama Red Cross Hospital, Nagahama, Shiga 526,
*The Third Department of Internal Medicine, Gifu University
School of Medicine, Gifu 500, **Takayama Red Cross Hospital,
Takayama, Gifu 506, and ***Sohgo Biomedical Laboratories,
Kawaguchi, Saitama 350, Japan

Abstract. In an attempt to investigate the clinical significance of anti-pituitary antibodies in patients with hypopituitarism, anti-pituitary antibody in plasma was examined in 10 such patients (7 cases of isolated ACTH deficiency, 1 of partial hypopituitarism, and 2 of Sheehan's syndrome), on two or three occasions with an interval of more than 6 months (longitudinal study). In a total of 16 relatives of these 4 patients (2 cases of Sheehan's syndrome, one in each of partial hypopituitarism and isolated ACTH deficiency) and one patient not involved in the longitudinal study, anti-pituitary antibodies were also examined (family study). Anti-pituitary antibodies reacting with rat pituitary cytoplasmic antigens (pituitary cell antibodies: PCA) and pituitary cell surface antibodies (PCSA) reacting with GH3 cells and/or Ait-20 cells were measured with indirect immunofluorescence. The longitudinal study revealed the disappearance of antibodies in 3 patients, 2 PCA positive and one both PCA and PCSA positive. In 3 patients, altered antibody titers or a newly appearing antibody were found during the follow-up period. In 4 patients, the pituitary antibodies were negative during the follow-up periods. Of 16 family members studied, positive PCA was found in 3 members (2 in the families of patients with PCA positive Sheehan's syndrome, and 1 in the family of the patients with PCA positive partial hypopituitarism). Positive PCSA was found in 4 members (one in each of families of patients with partial hypopituitarism and isolated ACTH deficiency and of two cases of Sheehan's syndrome), and weakly positive PCSA was found in one family member of a patient with PCA positive Sheehan's syndrome. The longitudinal study indicated that anti-pituitary antibodies may disappear during the course of the disease. A family study suggested that some types of hypopituitarism might involve an autoimmune process with a hereditary background for their pathogenesis.

(Endocrinol Jpn 38: 121–129, 1991)

ADENOHYPOPHYSITIS has been reported to be one of the causes of hypopituitarism [1–13]. The involvement of the autoimmune process in the pathogenesis has been suspected on the basis of histological and experimental findings [14], by demonstrating circulating anti-pituitary antibody-like substances [7], or the association with other autoimmune endocrine disorders [1, 3, 6, 10]. Furthermore, antibodies to the anterior pituitary cells (anti-pituitary antibody) were demonstrated in some cases of Sheehan's syndrome [15] and several types of anterior pituitary hypofunction [16]. However, the pathophysiology of the aforementioned disorder, and the physicochemical properties of anti-pituitary antibody remain unclear. To define the clinical or pathophysiological significance of the anti-pituitary antibody in hypopituitarism, its presence in the serum of the patients as well as in their relatives should be monitored for long periods. In the present study,
both antibodies to pituitary cytoplasmic antigen (PCA), and to pituitary cell surface antigen (PCSA) were followed up in the patients with hypopituitarism and also examined in their healthy family members.

Materials and Methods

Seven cases of isolated ACTH deficiency (Case 1 to Case 7), one case of partial hypopituitarism (Case 8), and 3 cases of Sheehan’s syndrome (Case 9 to Case 11) were studied (Table 1).

Longitudinal study: The presence of anti-pituitary antibodies was studied on more than two occasions over 6 months in 10 patients (Case 1 to Case 10).

Family study: In a total of 16 relatives of 5 patients (Cases 6, 8, 9, 10 and 11) endocrine functions and the presence of anti-pituitary antibody were examined.

Assay of anti-pituitary antibody: Anti-pituitary antibodies reacting with pituitary cytoplasmic antigens (pituitary cell antibodies: PCA), and anti-pituitary antibodies reacting with pituitary cell surface antigens (pituitary cell surface antibody: PCSA) were assayed by immunofluorescence methods in Sohgo Biomedical Laboratories (Saitama, Japan), as reported previously [17–19]. Briefly, unfixed 5 μm cryostat pituitary sections obtained from Wistar male rats (7 weeks old) were used for PCA assay. Aliquots of sera from patients, with or without absorption with rat liver acetone powder, were added to these sections on slides and kept for 30 min at room temperature (about 20°C) in a humidified chamber. They were washed twice with phosphate buffered saline (PBS) (pH 7.4). Twenty-five μl of biotinylated anti-human IgG solution (0.5 mg/ml PBS, Vector Lab. Inc., CA, USA) was then added to the sections and the slides were kept for 30 min at room temperature. After washing twice with PBS (pH 8.0), 25 μl of FITC-labelled avidin D solution (0.167 mg/ml PBS, Vector Lab. Inc., CA, USA) was added to the sections and incubated at room temperature for 30 min. After washing 3 times with PBS (pH 8.0), the sections were examined under an incident fluorescence microscope. Sera known to be PCA positive, as well as sera from healthy blood donors, were assayed in parallel (Fig. 1). Each serum was tested on different sections of at least 2 rat pituitary glands.

For the study of PCSA, GH3 and AtT-20 cells were used. Heat inactivated sera, which were preabsorbed with rat liver acetone powder, were diluted 4 times (in the case of GH3 cells as antigens) or 10 times (in the case of AtT-20 cells as antigens) with Hank’s medium containing 4% BSA (medium A). The diluted sera (200 μl) and cell suspension (5×10⁴ cells/100 μl) were mixed and allowed to stand for 30 min at 4°C. After washing twice with medium A, 100 μl of FITC-labeled anti-human IgG solution (diluted 10 times with medium A) was added to these cell suspension and was kept for 30 min at 4°C. After again washing twice with medium A, the cell suspension was examined under an incident fluorescence microscope at 400 × magnification. A cell was taken to be fluorescence positive when it was surrounded by at least 4 fluorescence dots (Fig. 2). For the estimation of PCSA, we adopted the fluorescence

<table>
<thead>
<tr>
<th>Table 1. Clinical features of the patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>sex</strong></td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Case 1</td>
</tr>
<tr>
<td>Case 2</td>
</tr>
<tr>
<td>Case 3</td>
</tr>
<tr>
<td>Case 4</td>
</tr>
<tr>
<td>Case 5</td>
</tr>
<tr>
<td>Case 6</td>
</tr>
<tr>
<td>Case 7</td>
</tr>
<tr>
<td>Case 8</td>
</tr>
<tr>
<td>Case 9</td>
</tr>
<tr>
<td>Case 10</td>
</tr>
<tr>
<td>Case 11</td>
</tr>
</tbody>
</table>
cell score. This score is the number of fluorescence positive cells in 100 cells counted under a microscope. With the sera from 10 healthy subjects, scores for GH3 and AtT-20 cells were less than 22% and 6% respectively, which was defined to be negative. Because of a relatively large coefficient of variation (about 30%), sera of which the scores were more than 35% and 15% were defined as having antibodies positive for GH3 and AtT-20 cells, respectively. Antibodies positive for scores between positive and negative were defined to be weakly positive. Both PCA and PCSA were negative in 25 normal controls [17–19].

Hormone assays: Plasma levels of various hormones were measured with the following commercially available radioimmunoassay kits: plasma GH (HGH RIA Kit II, Dainabot, Tokyo), TSH (TSH RIA BEAD II, Dainabot, Tokyo), PRL (SPAC S Prolactin Kit, Daiichi Radioisotope, Tokyo), ACTH (ACTH II Kit, CIS Gif-sur-Yvette, France), LH (LH Kit Daiichi, Daiichi Radioisotope, Tokyo), FSH (FSH Kit Daiichi, Daiichi Radioisotope, Tokyo), and cortisol (SPAC Cortisol Kit II, Daiichi Radioisotope, Tokyo).

Anterior pituitary function was assessed with the peak values of plasma TSH and PRL in response to TRH (500 μg, Takeda, Osaka, Japan), LH and FSH to LH-RH (100 μg, Tanabe, Osaka, Japan),
ACTH and cortisol to human corticotropin-releasing factor (CRH) (100 μg, Peptide Institute, Osaka, Japan) or the hypoglycemia induced by human actrapid insulin (0.05 IU/Kg of body weight, Novo), and GH to the infusion of arginine for 30 min (0.5 g/Kg of body weight, infused for 30 min) or to the insulin induced hypoglycemia. The blood samples were drawn before and at 15, 30, 60, 90 and 120 min after the injection of each substance.

Case 1: He had noticed malaise in October 1984. He showed no responses of plasma ACTH and cortisol to CRF, and normal responses of the other pituitary hormones to the pituitary stimulation tests. He was diagnosed as having isolated ACTH deficiency in July, 1985. PCA was positive in the sera obtained in July and September (2 weeks after the start of replacement therapy). It became negative in April, 1987. However, plasma levels of ACTH and cortisol were still undetectable in any sample during the CRF test performed in September, 1990.

Case 2: She noticed an episode of hypoglycemic attack in March, 1973, and was diagnosed as having isolated ACTH deficiency in August, 1984.

Fig. 2. Fluorescence positive GH, cells (upper) and AtT-20 cells (lower). (Magnification × 400). The cell was determined to be fluorescence positive when it was surrounded by at least 4 or more fluorescence dots.
PCA was positive on the first consultation (before the start of replacement treatment), and it became negative in February, 1985.

Case 3: He first noticed malaise in September, 1987, and was diagnosed as having isolated ACTH deficiency in October, 1987. PCA was negative and PCSA to AtT-20 cells was weakly positive in November, 1987 (1 month after the start of hormone replacement). PCA was positive and PCSA to AtT-20 cells was weakly positive in September, 1988. PCSA to GH3 cells and AtT-20 cells were positive in September, 1989.

Case 4: He had been suffering from malaise since April, 1985. Isolated ACTH deficiency was diagnosed in July, 1985. PCA was negative and PCSA reacting with AtT-20 cells was positive in November, 1986 (16 months after the diagnosis and start of the replacement). PCA was positive and PCSA to GH3 cells and AtT-20 cells in the sera obtained in May, 1988 changed to weakly positive. PCSA to AtT-20 cells was weakly positive in October, 1989.

Case 5: He had had anorexia and malaise since February, 1985. Isolated ACTH deficiency was diagnosed in March, 1986 and subsequently treated with hydrocortisone. Anti-pituitary antibodies were negative on two occasions (in September, 1986, and November, 1988).

Case 6: He had had anorexia, malaise and episodes of mental confusion since 1983 and isolated ACTH deficiency was diagnosed in July, 1984. Anterior-pituitary antibodies were negative in September, 1986 and November, 1988.

Case 7: He had been suffering from anorexia and depression since August, 1981 and isolated ACTH deficiency was diagnosed in April, 1982. Anti-pituitary antibodies were negative in January, 1987, and October, 1988.

Case 8: Cranial magnetic resonance imaging revealed empty sella. Endocrinological examination showed low response of plasma GH to arginine, and relatively low response of plasma TSH to TRH. Both PCA and PCSA reacting with GH3 cells and AtT-20 cells were positive in December, 1986 when she was diagnosed as having partial hypopituitarism, and they became negative in August, 1988.

Case 9: She had stillbirth with excessive genital bleeding in November, 1973. PCA was positive and PCSA was negative in September, 1986 when Sheehan’s syndrome was diagnosed. PCA was positive, and PCSA reacting with both GH3 cells and AtT-20 cells was weakly positive in November, 1988.
Case 10: She had noticed amenorrhea and loss of pubic hair after postpartum genital bleeding in December, 1968. After several episodes of unconsciousness she was diagnosed as having Sheehan’s syndrome in August, 1985. Neither PCA nor PCSA was positive in November, 1986, or in May, 1988.

Family study (Fig. 4, Table 2)

Case 6: Neither PCA nor PCSA was positive in the patient. PCSA to AtT-20 cells was positive in one of his 3 children. Anterior pituitary function tests performed in the son with positive PCSA revealed normal responses of plasma PRL to TRH, plasma LH and FSH to LH-RH, and plasma GH to insulin induced hypoglycemia. But plasma TSH response to TRH and serum cortisol response to hypoglycemia were slightly lower than the normal ranges.

Case 8: Both PCA and PCSA to GH3 cells and AtT-20 cells were positive in the patient at the first examination and in one of her 3 children.

Case 9: PCA was positive in the patient at the first examination. Her elder brother and one of his 2 sons were positive for PCA. Although she was initially negative for PCSA to AtT-20 cells, she became weakly positive at the second examination. It was also found to be weakly positive in one of her 2 elder sisters. Anterior pituitary functions were within normal limits in the PCA positive brother with slightly low response of plasma GH to arginine.

Case 10: Neither PCA nor PCSA was positive in the patient. These antibodies were also negative in one of the 3 siblings and in a son. PCSA reacting with AtT-20 cells and PCA were positive in a daughter. Her anterior pituitary functions were within normal limits. Magnetic resonance imaging of her pituitary was normal.

Case 11: Neither PCA nor PCSA was positive in the patient. PCSA to AtT-20 cells was positive in one of her 2 sons. His anterior pituitary functions were within normal limits, except low cortisol and ACTH responses to CRF and insulin induced hypoglycemia.

In all the above cases with positive PCA, however, this antibody was absorbed with rat liver acetone powder.

Fig. 4. Results of family study. ▶ Presented patients. Results of their initial examination are shown.
Table 2. Anterior pituitary function tests in anti-pituitary-antibody positive relatives of the presented patients

<table>
<thead>
<tr>
<th>Test</th>
<th>Plasma hormone assayed</th>
<th>Son of *6 Basal</th>
<th>Maximal</th>
<th>Brother of *9 Basal</th>
<th>Maximal</th>
<th>Daughter of *10 Basal</th>
<th>Maximal</th>
<th>Son of *11 Basal</th>
<th>Maximal</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRH test</td>
<td>TSH (mU/L)</td>
<td>0.5</td>
<td>4.6</td>
<td>2.8</td>
<td>12.8</td>
<td>0.9</td>
<td>14.3</td>
<td>1.6</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>PRL (ng/L)</td>
<td>10.9</td>
<td>35.6</td>
<td>4.6</td>
<td>27.6</td>
<td>4.8</td>
<td>47.8</td>
<td>12.7</td>
<td>32.7</td>
</tr>
<tr>
<td>LH-RH test</td>
<td>LH (IU/L)</td>
<td>12.2</td>
<td>65.8</td>
<td>19.9</td>
<td>323.8</td>
<td>6.7</td>
<td>25.7</td>
<td>9.9</td>
<td>24.4</td>
</tr>
<tr>
<td></td>
<td>FSH (IU/L)</td>
<td>7.6</td>
<td>17.2</td>
<td>18.8</td>
<td>88.5</td>
<td>7.5</td>
<td>11.6</td>
<td>6.8</td>
<td>67.9</td>
</tr>
<tr>
<td>Insulin induced</td>
<td>Nadireglucose level</td>
<td>1.74</td>
<td>2.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypoglycemia test</td>
<td>GH (µg/L)</td>
<td>&lt;1.5</td>
<td>20.7</td>
<td>&lt;1.5</td>
<td>6.7</td>
<td>&lt;1.5</td>
<td>35.1</td>
<td>342</td>
<td>400</td>
</tr>
<tr>
<td>CRF test</td>
<td>Cortisol (nm)</td>
<td>248</td>
<td>353</td>
<td>356</td>
<td>560</td>
<td>3.10</td>
<td>11.2</td>
<td>1.87</td>
<td>6.03</td>
</tr>
<tr>
<td></td>
<td>ACTH (pM)</td>
<td>361</td>
<td>612</td>
<td>351</td>
<td>433</td>
<td>15.1</td>
<td>42.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In summary, PCA was positive in 2 cases (#1 and 2) and both PCA and PCSA were positive in one (#8) before therapy. They became negative after the replacement therapy in the former 2 cases (#1 and 2) and without therapy in the latter (#8). Only one PCA positive patient (#9) before therapy developed PCA positive and weakly positive PCSA to AtT-20 cells and GH<sub>3</sub> cells after the replacement. One case (#3) with weakly positive PCSA to AtT-20 cells at the first examination showed PCA positive and weakly positive PCSA to GH<sub>3</sub> cells and AtT-20 cells at the second testing, and PCSA to GH<sub>3</sub> and AtT-20 cells was positive at the third examination. The other case (#4) with positive PCSA to AtT-20 cells at the first examination showed PCA positive and weakly positive PCSA to GH<sub>3</sub> cells and AtT-20 cells at the second testing and weakly positive PCSA to AtT-20 cells at the third examination. In 4 cases (#5, 6, 7 and 10), both PCA and PCSA were negative at the first and the second examinations. All 4 cases (#1, 2, 8 and 9) examined before treatment showed positive PCA or positive PCA and PCSA. In 6 cases examined after the replacement therapy, 2 (#3 and 4) were weakly positive and positive for PCSA, respectively. In the other 4 cases (#5, 6, 7 and 10), the antibodies were not detected. Out of 16 relatives in 5 cases with hypopituitarism, 2 were PCA positive, 3 were PCSA positive to AtT-20 cells, one was PCSA weakly positive to AtT-20 cells and one was both PCA and PCSA positive to GH<sub>3</sub> cells and AtT-20 cells. Furthermore, among the 6 first-degree relatives, both or either PCA or PCSA was positive in 4 (67%). On the other hand, out of 9 second or third degree relatives, 3 (33%) were positive for PCA or PCSA.

Discussion

Although many cases of hypopituitarism with anti-pituitary antibodies have been reported, there is no direct evidence that hypopituitarism is caused by adenohypophysitis. Although our results showed high prevalence of anti-pituitary antibody in the sera of patients with hypopituitarism, it is not direct evidence that hypopituitarism resulted from an autoimmune disorder, but indicated the possible participation of an autoimmune mechanism in its pathogenesis.

Fundamental disagreement has been seen in the methods for detecting anti-pituitary antibody which have been based on the indirect immunofluorescence technique [20]. The choice of pituitary tissue for the antigen has been the most controversial issue. Although fresh human pituitary may be the best source for the assay, there is great difficulty in obtaining the tissue. Human postmortem pituitaries do not always give clinically corresponding results in addition to the difficulty in routine supply; and fetal glands, on the other hand, have an irrelevant limiting factor [21]. The use of animal pituitaries or a cultured cell line is more practical if it yields good reproducibility and clinically correlated results. The high sensitivity and organ specificity of the present methods used for the detection of antibodies reacting with pituitary cell cytoplasmic antigens and with pituit-
ary cell surface antigens were discussed previously [17, 18] and showed that the present method was applicable for clinical practice. Serum anti-pituitary antibody, as measured by our method, was not found in normal subjects [17–19]. In our previous study [17], these antibodies were detected only in patients with isolated ACTH deficiency, IDDM, and chronic thyroiditis. Preabsorption of sera by rat liver acetone powder in our method excludes organ-non-specific reactivity in detecting PCSA. Because of spontaneous fixation of human immunoglobulins on human ACTH-producing cells with their Fc-receptors, the detection of a specific autoantibody to the cells has been technically difficult [16]. There was little non-specific binding of immunoglobulins to GH3 cells or AtT-20 cells (below 29%, and 6%, respectively) with our method. Organ-non-specific reactivity in the PCA assay makes it difficult to evaluate our results. This antibody in PCA positive patients was absorbed with rat acetone powder. Despite these problems, the fact that PCA was negative in the normal control [17] indicates the pathophysiological significance of PCA. Anyway, standardization of the measurement of PCA or PCSA will be required to improve the specificity.

We studied anti-pituitary antibodies in patients with 3 types of hypopituitarism (isolated ACTH deficiency, partial hypopituitarism with empty sella, Sheehan’s syndrome). There has been reported a diversity of clinical findings in Sheehan’s syndrome: Sheehan’s syndrome presenting panhypopituitarism or partial hypopituitarism, Sheehan’s syndrome with or without massive genital bleeding [22], or isolated ACTH deficiency after postpartum hemorrhage (atypical Sheehan’s syndrome) [23]. It remains unknown whether these disorders have a common pathogenesis including autoimmune dysfunction. The present results showing that anti-pituitary antibodies are frequently detected in patients with various clinical types of hypopituitarism suggests, however, the possibility of a common immunological abnormality.

The disappearance of anti-pituitary antibody was observed in 3 patients with hypopituitarism. No improvement of pituitary function was observed in one such patient. This observation is comparable with that of islet cell antibody in IDDM in which the detection rate falls with time after the onset. Although PCA or PCSA persisted during the follow-up period in 3 other patients, the titers of the antibodies changed, which may be partly due to large coefficients of variation in the indirect immunofluorescence method. We also could not exclude the possibility of the disappearance of the antibody before the examination in patients with anti-pituitary antibody negative hypopituitarism.

The family study revealed that anti-pituitary antibodies were often detected in the family members. Mirakian et al. [24] reported that pituitary cell antibodies were found in patients with juvenile onset diabetes and their families. Our results also suggest that the autoimmune abnormality in patients with hypopituitarism may have hereditary background, and the appearance of anti-pituitary antibodies would not be the result of pituitary destruction. Higher prevalence in first degree relatives than in other relatives may indicate such a possibility. However, there have been few reports on familial onset of hypopituitarism including Sheehan’s syndrome. On the other hand, pituitary antibody is detectable in some of patients with empty sella [19]. Furthermore, a case of hypophysitis with autoantibody to prolactin secreting cells whose anterior pituitary functions were within the normal range except hyperprolactinemia was reported [25]. PCA or PCSA may be a marker of pituitary inflammation, because the antibodies were detected in lymphoid adenohypophysitis [7, 25] and in certain cases disappeared spontaneously with decreased inflammation. The measurement of PCA or PCSA is significant in monitoring pituitary inflammation. To our knowledge, there is no reason to assume that anti-pituitary antibodies are directly cytotoxic to pituitary cells. Nicholas et al. [26] very recently suggested that anti-pituitary antibody could play a pathogenic role by either inhibiting a POMC-processing enzyme or initiating an antibody-dependent cellular cytotoxicity reaction in a patient with isolated ACTH deficiency. A more detailed experimental and clinical study including prospective follow-up of healthy family members with positive anti-pituitary antibodies for long periods will help to throw light on these issues.

Acknowledgements

The authors appreciate the review of the manu-
script by Dr. L. B. Mercado-Asis. This work was supported in part by research grants from the Intractable Disease Division, Public Health Bureau, Ministry of Health and Welfare, “Disorders of Hypothalamo-Pituitary Gland” and “Disorders of Adrenal Gland”, Japan.

References


