HETEROGENEITY OF ANTERIOR PITUITARY CELL ANTIBODIES DETECTED IN INSULIN-DEPENDENT DIABETES MELLITUS AND ADRENOCORTICOTROPIC HORMONE DEFICIENCY

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SUMMARY A sensitive assay method for pituitary cell antibodies (PitCA) was established by a biotin/avidin system using rat pituitary. Results in 24 cases of insulin-dependent diabetic patients and 10 cases of 21 adrenocorticotropic hormone (ACTH) deficient patients were positive for autoantibodies to anterior pituitary cell cytoplasm. PitCA observed in the sera of insulin-dependent diabetic patients were suspected of being pituitary specific and independent with islet cell antibodies (ICA) and islet cell surface antibodies (ICSA). In the sera of ACTH deficient patients, PitCA were frequently absorbed with liver acetone powder. Populations of insulin-dependent diabetes mellitus (IDDM) are almost equal in males and females. A total of 16 cases of ACTH deficient patients were female. These results suggest that heterogenous PitCA are involved in the sera of those patients with IDDM and ACTH deficiency.

Key words: Anterior pituitary cell, antibodies, insulin-dependent diabetes, ACTH deficiency

INTRODUCTION

Pituitary cell antibodies (PitCA) were observed in 16.6% of insulin-dependent diabetic patients by conventional immunofluorescence assay using cryostat sections of human pituitary glands (1, 2). Onodera and co-workers (3) reported on an animal model in which the reovirus Type 1 infection had produced a disease characterized by hyperglycaemia and retardation of growth. They observed virus-infected cells in both the pancreas and pituitary gland. Autoantibodies that react with antigens in pancreatic islets, anterior pituitary and gastric mucosa were found in the sera of these animals. Furthermore, treatment of reovirus infected mice with rabbit anti-mouse lymphocyte serum inhibited the appearance of antibodies which reacted to pancreatic islets and anterior pituitary cells (4).

Deficiency of ACTH and gonadotropin associated with normal thyrotropic function was first reported by Maddock et al. (5) and Myers et al. (6) in 1953. Recently, isolated ACTH deficiency was considered to be caused by (a) a decrease in corticotropin releasing factor (CRF), (b) selective destruction of ACTH secreting pituitary cells, or (c) refractoriness of pituitary cells to CRF. In the case of (b), an autoimmune process was suggested as one of the pathogenic factors (7). Furthermore, in overt ACTH deficiency, PitCA was frequently observed with rat or guinea pig pituitary gland (8).

In the present report, a sensitive assay method for PitCA was established by a biotin/avidin detection technique (9) using tissue antigens of rat pituitary. Characteristics of PitCA in the sera of the patients afflicted with IDDM and ACTH deficiency were studied.

Abbreviations: PitCA—pituitary cell antibodies, ACTH—adrenocorticotropic hormone, ICA—islet cell antibodies, ICSA—islet cell surface antibodies, IDDM—insulin-dependent diabetes mellitus, CRF—corticotropin releasing factor, LMAb—liver membrane antibodies.
MATERIALS AND METHODS

Sera

Sera were collected from 81 patients with IDDM, 21 patients with ACTH deficiency and 24 healthy subjects who served as controls. Aliquots of the same sera were absorbed with rat liver acetone powder (100 mg/ml serum) at 4°C for 24 hr. An absorption study with rat kidney, spleen and rabbit muscle acetone powder was also performed.

PitCA Assay by Biotin/Arachid System

Rat pituitary gland was obtained from Wistar male rats (7 weeks old). Unfixed 5 μm cryostat sections were used for the study. Undiluted sera of diabetic, ACTH deficient patients and healthy subjects were tested. Aliquots of serum (25 μl), with or without absorption with rat liver acetone powder, were added to sections on slides and kept for 30 min at r.t., in a humidified chamber. They were then washed twice with PBS (pH 7.4), 25 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 r.t. 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 r.t. for an additional 30 min. After 3 washings with PBS (pH 8.0), the sections were examined under an incident fluorescence microscope (Olympus BH-2). Sera known to be PitCA positive, drawn from patients with IDDM and/or ACTH deficiency, as well as sera from healthy blood donors, were assayed in parallel for control. Each serum was tested on different sections of at least 2 rat pituitary glands.

ICA and ICSA Assay

ICA was assayed by indirect immunofluorescence assay using monkey pancreas cryostat sections (BD-duX INC., NJ, USA) (10). ICSA was assayed by indirect rosette assay using dispersed rat pancreas islet cells and protein A-labelled sheep red blood cells (11).

Liver Membrane Antibodies (LMAb) Assay

LMAb was assayed by indirect immunofluorescence assay using cryostat sections of rat liver (12).

For statistical analysis, the χ²-test was used.

RESULTS

Figure 1 shows the PitCA in the sera from the patients with IDDM and ACTH deficiency. PitCA was present in 24 of 81 patients with IDDM (table 1). After absorption of the sera with rat liver acetone powder, 18 cases in 24 of PitCA-positive sera (75.0%) retained reactivities to anterior pituitary cell cytoplasm. The effect of absorption of sera with rat kidney, spleen and rabbit muscle acetone powder was further examined in 4 patients with IDDM, who were positive in reaction to PitCA. In each case, immunofluorescence in the cytoplasm of anterior pituitary cells was still observed (data not shown).

PitCA with ACTH deficiency was examined in the sera obtained from 21 ACTH deficient patients, and 10 or 2 sera were positive to PitCA. Only 2 sera out of these 10 (20.0%) reacted slightly to pituitary cells after absorption of sera with rat liver acetone powder. The other 8 specimens did not retain reactivity (table 1). Interest was also observed in some cases of subjects with ACTH deficiency, LMAb were also observed (table 2, fig. 2). LMAb were not observed in the group of ICA-, ICSA-negative, PitCA positive IDDM patients (data not shown).

Sera from patients with IDDM were simultaneously tested for ICA and ICSA (table 3). The percentage of PitCA-positive cases in the ICA-positive group was 6 (6/20), and was significantly higher than that of ICA-negative group (6% 61) (p < 0.005). The significant difference in the prevalence of PitCA between ICSA-positive and ICSA-negative groups was also observed. PitCA were also detected in the group of ICA-negative and ICSA-negative cases (12% 11/52).

As shown in Table 4, the populations of male and female insulin-dependent diabetic patients were almost equal. Of the ACTH deficient patients, 16 cases...

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Before absorption (A)</th>
<th>After absorption (B)</th>
<th>B/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDDM</td>
<td>81</td>
<td>24 (29.6%)</td>
<td>18 (22.2%)</td>
<td>0.75</td>
</tr>
<tr>
<td>ACTH deficiency</td>
<td>21</td>
<td>10 (47.6%)</td>
<td>2 (9.5%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Healthy control</td>
<td>24</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

* Absorption with rat liver acetone powder.

Fig. 1. Fluorescence in the cytoplasm of rat anterior pituitary (Magnification × 400). PitCA-positive serum from a patient with IDDM. The staining pattern of the serum from a patient with AC deficiency is almost identical with that from insulin-dependent diabetic patient. The serum was preabsorbed with rat liver acetone powder,
female and 5 cases were male. A female predominance of PitCA in ACTH deficiency was suggested by our results.

**Discussion**

Antibodies reactive not only to pancreatic islet cells (ICA, ICSA) but also to other endocrine cells were often detected in IDDM. They attract special interest because they have a close relation to pathogenesis of IDDM. Mirakian and co-workers reported that within a 1-yr duration of IDDM, 16.6% of the sera stained anterior pituitary cytoplasm, and 37% of the sera in ICA-positive relative to ICSA were PitCA-positive. Among these cases, 33% became diabetic during the first 3-yr follow-up period (2). Recently, PitCA were also detected in the sera of patients with ACTH deficiency using human and rodent pituitary glands (8).

In the present study, we assayed PitCA with a biotin/avidin system using rat pituitary gland. With this assay method, the prevalence of PitCA in IDDM and ACTH deficient patients was investigated. 24 of the 81 IDDM patients were positive for PitCA, and the prevalence of PitCA was comparable to ICA and ICSA. About half of the sera of the patients with ACTH deficiency were positive to PitCA. In the sera of healthy subjects, PitCA were not detected. It seemed that organ specific PitCA was considerably different between cases of IDDM and ACTH deficiency. PitCA in IDDM patients were suspected to be organ-specific and independent of ICA and ICSA. However, PitCA in subjects with ACTH deficiency were markedly absorbed by liver tissue. In samples which retained reactivities to pituitary cells after absorption, immunofluorescence was faint and quite reduced in density compared with pre-absorbed sera. Interestingly, LMAb were also observed in some cases of ACTH deficiency. Antibody-like reactivities specific to the cell surface membrane of anterior pituitary cells were frequently observed in the sera of IDDM and ACTH deficient patients. These antibodies were often absorbed to a considerable degree by liver tissue. Their organ specificity and clinical significance, therefore, still need to be elucidated.

As pathogenic factors of IDDM, virus infection (1) and autoimmune mechanism (14) have been reported. The cases of patients with virus-induced diabetes mellitus were microorganisms were extracted from the pancreas and shown to induce insulitis in mice (13). These microorganisms have not yet been defined in the anterior pituitary of diabetic patients, but hypopituitarism associated with diabetes has been reported (15, 16). Furthermore in an animal model, Onodera et al. observed virus-infected cells in both the pancreas and pituitary gland of reovirus-infected diabetic mice (3). These reports, and the fact that PitCA were observed in the sera of IDE patients, independent of ICA or ICSA, suggest the possibility that such viruses attack and destroy the anterior pituitary gland, and also lead to the production of PitCA in the sera of IDDM patients.

In respect to isolated ACTH deficiency, there were pathogenic factors, i.e. (a) CRF deficiency, (b) selective

**Table 3** Relation of PitCA to ICA and ICSA

<table>
<thead>
<tr>
<th>ICA</th>
<th>ICSA</th>
<th>No. of patients</th>
<th>n (％)</th>
</tr>
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<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>8</td>
<td>1 12.5</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>12</td>
<td>5 41.7</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>9</td>
<td>1 11.1</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>52</td>
<td>11 21.2</td>
</tr>
</tbody>
</table>

**Table 4** Sex ratio and PitCA of IDDM and ACTH deficiency

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Sex</th>
<th>PitCA (+) Before absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDDM</td>
<td>81</td>
<td>39M</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42F</td>
<td>11</td>
</tr>
<tr>
<td>ACTH deficiency</td>
<td>21</td>
<td>5M</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16F</td>
<td>9</td>
</tr>
</tbody>
</table>

**Fig. 2** Fluorescence in the plasma membrane of rat liver (Magnification ×200): LMAb-positive serum from a patient with ACTH deficiency.
destruction of ACTH secreting pituitary cells and (c) refractoriness of pituitary cells to CRF. In the case of (b), destruction of ACTH secreting pituitary cells by autoimmune mechanism has been suggested (7,17-19). We detected PitCA in 10 cases out of 21 ACTH deficient patients. Autoimmune-mediated destruction of ACTH secreting cells was indicated in all such cases. In general, females were predominant in cases of autoimmune disease. In our own experience, 16 cases out of 21 ACTH deficient patients were female. Interestingly, ACTH deficiency was occasionally associated with Hashimoto's disease (17,18). These reports and rather weak organ specificity of PitCA observed in patients with ACTH deficiency suggest the possibility that PitCA may often be associated with thyroid antibodies and/or LMAAb in the sera of these ACTH deficient patients.

In conclusion, PitCA were observed in the sera of IDD and ACTH deficient patients, and the heterogeneity of these antibodies was thus suggested.

Further experiments on the role of pituitary cell cytoplasmic and surface membrane antibodies in relation to pathogenesis and clinical significance are now in progress in our laboratories.

REFERENCES