DETECTION OF ANTIBODIES TO ANTERIOR PITUITARY CELL SURFACE MEMBRANE WITH INSULIN DEPENDENT DIABETES MELLITUS AND ADRENOCORTICOTROPIC HORMONE DEFICIENCY

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SUMMARY Autoantibodies for anterior pituitary cell surface membrane (PitCSA) were assayed by immunofluorescence method using GH₃ cells (rat GH and prolactin secreting cell) and AtT-20 cells (mouse adrenocorticotrophic hormone secreting cell) as antigens. Out of 18 insulin dependent diabetic patients who were positive for antibodies to islet cell surface membrane (ICS), 3 cases (16-7%) were positive for antibodies to GH₃ cells and 12 cases (66-7%) were positive for antibodies to AtT-20 cells. Moreover, out of 18 insulin dependent diabetic patients who were negative for ICSA, 2 (11-1%) and 6 cases (33-3%) were positive for antibodies to GH₃ cells and AtT-20 cells, respectively. Among 5 adrenocorticotrophic hormone (ACTH) deficient patients, all of the sera were positive for antibodies to AtT-20 cells. These results suggested that PitCSA and ICSA have independent features, though both are closely related, and that PitCSA was one of the significant immunological markers often observed in the sera of the patients with insulin dependent diabetes mellitus (IDDM) and ACTH deficiency.

Key words: Pituitary cell surface membrane, GH₃ cell, AtT-20 cell, autoantibodies, insulin dependent diabetes mellitus, ACTH deficiency

INTRODUCTION

Pituitary cell antibodies (PitCA) have been observed in IDDM and ACTH deficient patients by conventional immunofluorescence assay (1,2) and a more sensitive biotin avidin system (3). In addition to autoantibodies reacting with anterior pituitary cytoplasmic antigens, autoantibodies to surface antigens on GH₃ cells (4) were also detected in virus-induced diabetic mice (5). In the previous report (3), we referred to the involvement of antibody-like reactivities specific to the surface membrane of anterior pituitary cells in the sera of IDDM and ACTH deficient patients.

In the present report, an assay method for PitCSA was established by immunofluorescence method using GH₃ and AtT-20 cells (6). A study on characteristics of PitCSA in the sera of the patients afflicted with IDDM and ACTH deficiency was carried out and the role of PitCSA on the pathogenesis and clinical significance is discussed.

MATERIALS AND METHODS

Sera

Sera were collected from 26 patients with IDDM, 5 patients with ACTH deficiency and 10 healthy subjects who served as controls. Sera were preabsorbed with rat liver acetone powder (100 mg/ml serum) at 4°C for 24 hr. Absorption studies with rat spleen, kidney, rabbit pancreas, brain and bovine thyroid gland acetone powder were also performed.

PitCSA Assay by Immunofluorescence Method

For the study of PitCSA, GH₃ and AtT-20 living cells were used as

Abbreviations: PitCSA—Pituitary cell surface antibodies; PitCA—Pituitary cell antibodies; ICSA—Islet cell surface antibodies; ACTH—Adrenocorticotropic hormone; IDDM—Insulin dependent diabetes mellitus; ICSC—Immune complex solubilizing capacity.
antigens. 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 × 10⁶ cells/100 µl) were mixed and stood for 30 min at 4°C. After washing twice with medium A, 100 µl of FITC-labeled anti-human IgG solution (diluted 10 times by medium A) was then added to the 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 (Olympus BH-2) at 400× magnification. A cell was considered to be fluorescent positive when surrounded by at least 4 fluorescence dots. For the estimation of PitCSA we adopted the fluorescence positive cell score. This score is the number of fluorescence positive cells relative to 100 cells counted under a microscope. With the sera from 10 healthy subjects, scores for GH₃ cells were all < 22%, and for AtT-20 cells < 6%. Therefore, sera of which scores were > 22 and 6% were estimated to be antibodies positive for GH₃ and AtT-20 cells, respectively.

**ICS Assay**

The procedure was modified from Lerumark's method (7). Islets of Langerhans were isolated from pancreas of rats by collagenase digestion and discontinuous density gradient centrifugation. Single cell suspension was obtained by further enzymatic (collagenase and hyaluronidase) digestion and mechanical shaking. ICSA was assayed by the immunofluorescence method using dispersed islet cell suspension as antigens.

**Preparation of Antisera**

A female New Zealand rabbit was immunized with living rat pancreas islet cells (1 × 10⁶ cells), once a week for a period of 3 months.

**RESULTS**

The immunofluorescence pattern of the antibodies positive sera to GH₃ cells and AtT-20 cells is shown in Figure 1a and b, respectively. At the dilution analysis of antibodies positive diabetic sera and negative control sera, the highest scores were observed at ×4 and ×10 dilution against GH₃ and AtT-20 cells, respectively (figs. 2a, b). Only a few weak fluorescent cells were observed with the sera from healthy controls. In subsequent experiments, therefore, 4 times and 10 times dilutions of sera were routinely used for GH₃ and AtT-20 cells, respectively.

The effect of absorption of sera with rat liver, spleen, kidney, rabbit pancreas, brain and bovine thyroid gland acetone powder on PitCSA was examined in 4 patients with IDDM who were positive in reaction to GH₃ cell antibodies (fig. 3), and 3 patients with IDDM who were positive to AtT-20 cell antibodies and 2 patients with ACTH deficiency who were positive to AtT-20 cell antibodies (fig. 4). In each of these cases, immunofluorescence reactivity remained, but liver acetone powder seemed to
Table 1  Autoantibodies to GH₃ and AtT-20 cells in IDDM, ACTH deficient patients and healthy subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>GH₃ cell (+) (%)</th>
<th>AtT-20 cell (+) (%)</th>
<th>GH₃ or AtT-20 cell (+) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDDM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICSA (+)</td>
<td>18</td>
<td>3 (16.7)</td>
<td>12 (66.7)</td>
<td>12 (66.7)</td>
</tr>
<tr>
<td>ICSA (-)</td>
<td>18</td>
<td>2 (11.1)</td>
<td>6 (33.3)</td>
<td>7 (38.8)</td>
</tr>
<tr>
<td>ACTH deficiency</td>
<td>5</td>
<td>1 (20.0)</td>
<td>5 (100.0)</td>
<td>5 (100.0)</td>
</tr>
<tr>
<td>Healthy control</td>
<td>10</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Fig. 3. Effect of absorption of sera with tissue acetone powder on immunofluorescence positive GH₃ cell score with 4 IDDM patients.

be the most effective material to absorb organ-non-specific reactivity. The result suggests that preabsorption of sera with liver acetone powder is essential to detect specific antibodies to GH₃ and AtT-20 cell surface membranes.

PitCSA with IDDM was examined in the sera obtained from 18 ICSA positive and 18 ICSA negative IDDM patients (table 1). In the ICSA positive group, 3 cases (16.7%) and 12 cases (66.7%) were positive for antibodies to GH₃ cells and AtT-20 cells, respectively. Three cases were positive for both cells. In the ICSA negative group, 2 cases (11.1%) and 6 cases (33.3%) were positive for antibodies to GH₃ and AtT-20 cells, respectively. One case was positive for both cells. PitCSA with ACTH deficiency was examined in the sera from 5 ACTH deficient patients, with one case and all 5 cases found positive for antibodies to GH₃ and AtT-20 cells, respectively. One case was positive for both. These autoantibodies were not detected at all in the sera from 10 healthy subjects.

Rabbit antisera to rat islet cells and In-111 cells strongly reacted to GH₃ and AtT-20 cells. The effect of absorption of antisera with various tissue acetone powders was examined with ×64 diluted antisera (table 2). In each case, immunofluorescence in the cell surface membrane was still observed.

Table 2  Effect of absorption of antisera to rat islet cells and In-111 cells on PitCSA. Antisera were diluted ×64 with medium A

<table>
<thead>
<tr>
<th>Tissue acetone powder</th>
<th>Antiserum to rat islet cell</th>
<th>Antiserum to In-111 cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GH₃</td>
<td>AtT-20</td>
</tr>
<tr>
<td>(-)</td>
<td>69.7</td>
<td>66.1</td>
</tr>
<tr>
<td>Liver</td>
<td>60.0</td>
<td>66.0</td>
</tr>
<tr>
<td>Spleen</td>
<td>60.0</td>
<td>62.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>60.0</td>
<td>33.6</td>
</tr>
<tr>
<td>Pancreas</td>
<td>48.6</td>
<td>32.0</td>
</tr>
<tr>
<td>Muscle</td>
<td>54.9</td>
<td>28.4</td>
</tr>
<tr>
<td>Brain</td>
<td>56.0</td>
<td>62.9</td>
</tr>
<tr>
<td>Lung</td>
<td>73.0</td>
<td>65.5</td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>49.6</td>
<td>59.0</td>
</tr>
</tbody>
</table>

Fig. 4. Effect of absorption of sera with tissue acetone powder on immunofluorescence positive AtT-20 cell score with 3 IDDM and 2 ACTH deficient patients.
DISCUSSION

In the previous report, we showed some characteristics of PitCRA relative to IDDM and ACTH deficiency using sections of rat pituitary gland (3). In the report we referred to the involvement of antibody-like reactivities specific to the cell membrane antigen of the anterior pituitary gland in the sera of IDDM and ACTH deficient patients. Tissue sections used as antigens were, however, inadequate to observe PitCRA, because the fluorescence of PitCRA interrupted the distinct observation of PitCRA. In the present study, we assayed PitCRA with an immunofluorescence method using living GH3 and AtT-20 cells. With this assay method, the disturbance of PitCRA interfering with observation of PitCRA could be excluded. As suspected previously (3), absorption of sera with liver acetone powder was essential to exclude organ-specific autoantibodies.

The incidence of PitCRA was considerably higher than that of PitCRA (3), and the incidence of autoantibodies to AtT-20 cells was higher than that of antibodies to GH3 cells with IDDM and ACTH deficiency. Furthermore, contrary to PitCRA (3), the absorption of the sera taken from ACTH deficient patients with liver acetone powder did not diminish PitCRA to AtT-20 cells. These data suggest the possibility that PitCRA may contribute to the pathogenicity of the autoimmune mechanism which causes the ACTH deficiency (8,9). The function of PitCRA to attack the anterior pituitary gland in IDDM remains to be ascertained. A limited number of cases in which hypopituitarism was associated with diabetes has been reported (10,11). It is noteworthy that in the sera of healthy subjects, immune complex solubilizing capacity (ICSC) (12) was supposed to be active enough to protect various organs from attack by autoantibodies. ICSC in the sera from IDDM and ACTH deficient patients might be related to the activity of PitCRA in attacking and destroying anterior pituitary cells.

It is important to elucidate whether or not the antigens different from islet cell surface membrane are involved in the anterior pituitary cell surface membrane. Part of the PitCRA in the sera of IDDM patients seemed to be organ-specific and independent of ICSC. Another portion(s) of antigens for PitCRA may be identical with that for ICSC, as reported by Boizard et al. (13). The data obtained from rabbit antisera as well as rat islet cell or In-111 cell suggest that part of the antigens for ICSC in identical with that for PitCRA. Circulating PitCRA of IDDM patients might be, however, somewhat different from passively immunized antisera to rat islet cells and In-111 cells in their reactivities to antigens.

Further experiments on the role of PitCRA observed in the sera from IDDM and ACTH deficient patients are now in progress in our laboratories.

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REFERENCES