Idiopathic central diabetes insipidus in children and young adults is commonly associated with vasopressin-cell antibodies and markers of autoimmunity

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Summary

Objectives Autoimmune targeting of hypothalamic-neurohypophysial structures in children and young adults with posterior pituitary and anterior pituitary dysfunction, as well as pituitary stalk involvement, are not yet completely understood.

Design We aimed to (1) evaluate the presence of circulating vasopressin-cell autoantibodies (AVPc-Abs) in young patients with central diabetes insipidus (CDI), (2) detect organ-specific autoantibodies as markers of autoimmunity, and (3) define the relationship between immune markers and neuroimaging findings.

Patients Twenty patients were evaluated at a median age of 16·3 years. Twelve patients had idiopathic CDI, six had Langerhans cell histiocytosis (LCH) and two had germinoma. AVPc-Abs were evaluated in 40 healthy children. Magnetic resonance imaging (MRI) of the hypothalamic-pituitary region was performed longitudinally in all subjects.

Measurements Circulating arginine vasopressin (AVP), protein tyrosine phosphatase (IA2), glutamic acid decarboxylase (GAD), 21-hydroxylase (21-OH), endomysium antibodies (EMA), parietal cell (PCA), thyroid peroxidase (TPO), thyroglobulin (TG) and TSH-receptor (TSHr) autoantibodies were evaluated.

Results Circulating AVPc-Abs were found in 15 patients (75%), nine with idiopathic CDI, four with LCH and two with germinoma; the pituitary stalk was involved in most of them. Five patients with idiopathic CDI showed a persistence of AVPc-Abs during follow-up and one became positive subsequently. Serum IA2 autoantibodies were demonstrated in 14 patients (70%) and 21-OH autoantibodies in three of them.

Conclusion In idiopathic CDI, circulating AVPc-Abs suggest an autoimmune involvement of the neurohypophysial system. The identification of AVPc-Abs in subjects who could have either idiopathic CDI or LCH or germinoma, however, indicates that AVPc-Abs cannot be considered a completely reliable marker of autoimmune CDI. Thus, close clinical and MRI follow-up are needed because AVPc-Abs may mask germinoma or LCH.

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Introduction

Central diabetes insipidus (CDI) is a heterogeneous condition characterized by the excretion of abnormally large volumes of dilute urine due to a deficiency of arginine vasopressin peptide (AVP). In many patients, especially children and young adults, it is caused by the destruction or degeneration of the neurones that originate in the supraoptic and paraventricular nuclei of the hypothalamus. The known causes of these lesions include germinoma or craniopharyngioma; Langerhans cell histiocytosis (LCH); local inflammatory, autoimmune or vascular diseases; trauma resulting from surgery or an accident; and in rare cases, genetic defects in AVP synthesis that are inherited as autosomal dominant or X-linked recessive traits. 1–11 Midline cerebral and cranial malformations are another possible cause of CDI. 12 However, 20–50% of cases are considered idiopathic. 1,13–15

An autoimmune process involving the hypothalamic-neuroendocrine AVP-producing cells leading to CDI was suggested in the early 1980s by Scherbaum and Bottazzo. 16 Vasopressin-cell autoantibodies (AVPc-Abs) were reported in 37% of subjects affected by idiopathic CDI, and in 6-3% of those with CDI associated with LCH, at a mean age of 34-9 years. These data were later confirmed by the same research group in six children with idiopathic CDI. 17 The fact that AVPc-Abs were recognized in only one-third of such patients seems to indicate either that they are subject to early disappearance or, possibly, that autoimmune T-cell local damage took place, not, however, necessarily associated with autoantibody formation. Indeed, the latter hypothesis was proposed by Imura et al., who used neuroimaging and histological diagnostic support to define cases of CDI of autoimmune/
inflammatory origin. They established that in 17 adult patients with CDI the entity ‘lymphocytic infundibulo-neurohypophysitis’ could have been caused by an autoimmune process affecting the posterior pituitary gland and infundibulum without anterior pituitary involvement.

More recently, a relationship between AVPc-Abs and clinical, immunological and radiological features has been demonstrated in 23% of a large cohort of subjects with CDI of different aetiologies at a mean age of 29±2 years. These findings demonstrate that autoimmune CDI most commonly affects female patients with associated autoimmune diseases who show pituitary stalk thickening at magnetic resonance imaging (MRI). Moreover, circulating AVPc-Abs have been reported in patients affected by autoimmune polyendocrinopathy, while pituitary stalk thickening and CDI have been described in the course of autoimmune polyglandular syndrome (APS) type 1.

The underlying process of pituitary stalk thickening in children and young adults with ‘idiopathic’ CDI and anterior pituitary hormone deficiency is not yet completely understood. In light of this, we decided: (1) to evaluate the presence of circulating AVPc-Abs in young patients with isolated CDI or with CDI associated with multiple pituitary hormone deficiency (MPHD) of different aetiologies, (2) to measure organ-specific autoantibodies as markers of autoimmunity, and (3) to define the relationship between immunological markers and neuroimaging findings.

**Materials and methods**

**Patients**

Twenty patients (10 males and 10 females) affected by permanent CDI of differing aetiology, ranging in age at the time of CDI diagnosis from 1±8 to 17 years (median 7~7±6 years), were reinvestigated at a median age of 16±3 years (range 5~7--27±8 years). Twelve patients had idiopathic CDI, six had LCH (the CDI diagnosis was confirmed at different time intervals compared to LCH) and two had germinoma. Four patients had isolated vasopressin deficiency and the remaining 16 had additional anterior pituitary hormone deficits. Six had GH deficiency and 10 had MPHD. The main clinical characteristics of the patients are summarized in Tables 1 and 2. All patients were being treated with antidiuretic hormone (arginine vasopressin; DDAVP), two or three times daily, either intranasally or orally. Anterior pituitary hormone deficiencies were being treated appropriately. The study protocol was approved by the appropriate review boards, and written informed consent was obtained from the patients or their parents or guardians.

**Posterior pituitary function**

The diagnosis of CDI was based on a history of polyuria and polydipsia, the results of a physical examination, laboratory evidence of AVP deficiency, and imaging studies of the brain and pituitary gland. When possible, cases were further classified according to the probable cause of CDI, including LCH and intracranial tumour (biopsy-proven). All remaining cases were considered idiopathic. The diagnosis of CDI was confirmed in all patients after a water deprivation test performed between midnight or 0830 and 1530 h followed by a DDAVP trial as described previously. A ratio of urinary osmolality to plasma osmolality of 1:0 or less was taken to indicate the presence of complete CDI, and a ratio of more than 1:0 but less than 1:4 was taken to indicate partial CDI. Serum sodium, plasma and urinary osmolality or specific gravity, and plasma immunoreactive AVP were measured at time 0 and at the end of the test. At the latter time point, plasma AVP concentrations in the normal subjects ranged from 2 to 5 pg/ml (1:8-4:6 pmol/l). The patients with thickened pituitary stalk underwent plasma and cerebrospinal fluid (CSF) evaluation of specific markers of germinoma. The patients with idiopathic CDI underwent skeletal surveys to rule out LCH as the cause.

**Anterior pituitary function**

Anterior pituitary function was assessed in all patients, both at the time of diagnosis and at the follow-up. Serum GH was measured before and 30, 60, 90 and 120 min after the administration of arginine (0±5 g/kg of body weight, intravenously over a period of 30 min), insulin (0±1 IU/kg, given intravenously) or levodopa (500 mg/m² of body-surface area, given orally). Patients with serum GH concentration of less than 10 µg/l and a deceleration in rate of growth were considered to have GH deficiency. The pituitary–thyroid axis was assessed every 6–12 months by measuring serum FT4, FT3 and TSH. Hypothyroidism was defined as a low or low-normal serum TSH concentration and low serum FT4, FT3 concentration. Plasma ACTH and serum cortisol were measured in the morning at presentation and every 6–12 months thereafter. ACTH deficiency was defined by either a serum cortisol concentration of less than 100 nmol/l (3±6 µg/dl) in the morning or peak serum cortisol concentration of less than 550 nmol/l (20 µg/dl) during insulin-induced hypoglycaemia.

Serum FSH and LH were measured before and 30, 60 and 120 min after the intravenous administration of 100 µg/m² GnRH in patients who were thought to have hypogonadotrophic hypogonadism. Hypogonadism was diagnosed in boys and girls who had no pubertal development and no increase in FSH and LH in response to GnRH. Ultrasonography was used to identify female patients with a prepubertal uterus. Subjects with MPHD were receiving conventional replacement therapy for pituitary deficits: 1~thyroxine 75–200 µg/day, hydrocortisone 10–20 mg/day in two to three separate doses, testosterone enanthate 150–250 mg intramuscularly every 2 or 3 weeks for males, and ethinyloestradiol (first 21 days, 5~10 µg/day orally, n = 2) or transdermal 17β-oestradiol patches (25–50 µg/day, n = 8) with medroxyprogesterone acetate (5–10 mg, day 12 to day 21) for females.

**Imaging studies**

MRI scans were obtained for all patients at presentation and every 3–6 months for the first 3–5 years, as well as at the time of the study protocol. MRI studies were performed with the spin–echo technique and use of a 1:5 T superconductive unit (Magnetom SP, Siemens, Erlangen, Germany). Sagittal and coronal, T1-weighted images (time repetition (TR)/echo time (ET), 310/15 ms, three acquisitions, 3-mm-thick sections, 256 x 256 matrix, and 20 cm field of view),
Table 1. Clinical features, pituitary stalk (PS) phenotype, serum AVPc-Abs and organ-specific Abs in idiopathic CDI

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age at diagnosis (years)/Sex</th>
<th>Age at study (years)</th>
<th>Disease duration (years)</th>
<th>AP hormone deficiencies</th>
<th>AVPc-IgG/IgA (titre)</th>
<th>I2A Abs (U/ml)</th>
<th>Organ-specific Abs (U/ml)*</th>
<th>PS at first MRI (mm)</th>
<th>Abs follow-up (years)</th>
<th>AVPc-IgG/IgA follow-up (titre)</th>
<th>I2A Abs follow-up (U/ml)</th>
<th>PS at follow-up MRI (mm)</th>
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<td>1</td>
<td>5·6/M 16·7</td>
<td>11·1</td>
<td>GH</td>
<td>1 : 64</td>
<td>0·57 (−)</td>
<td>Neg</td>
<td>4·6</td>
<td>0·3</td>
<td>NE</td>
<td>NE</td>
<td>4·6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7·4/F 12·2</td>
<td>4·8</td>
<td>−</td>
<td>1 : 16</td>
<td>1·49 (+)</td>
<td>Neg</td>
<td>4·3</td>
<td>0·3</td>
<td>NE</td>
<td>1·49</td>
<td>2·1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8·8/M 20·6</td>
<td>11·8</td>
<td>GH</td>
<td>1 : 32</td>
<td>1·50 (+)</td>
<td>Neg</td>
<td>4·9</td>
<td>0·7</td>
<td>1·16 (?)</td>
<td>1·5</td>
<td>2·2</td>
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<tr>
<td>4</td>
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<td>6·3</td>
<td>GH/ACTH</td>
<td>1 : 4</td>
<td>0·12 (−)</td>
<td>Neg</td>
<td>5·3</td>
<td>1·2</td>
<td>NE</td>
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<td>3·2</td>
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<tr>
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<td>1·32 (?)</td>
<td>1·32</td>
<td>2·7</td>
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<tr>
<td>6</td>
<td>7·7/F 7·8</td>
<td>0·1</td>
<td>−</td>
<td>1 : 8</td>
<td>1·41 (+)</td>
<td>Neg</td>
<td>1·8</td>
<td>0·7</td>
<td>1·16 (?)</td>
<td>1·9</td>
<td>4·8</td>
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<td>1 : 8</td>
<td>1·45 (+)</td>
<td>Neg</td>
<td>5·3</td>
<td>1·4</td>
<td>1·32 (?)</td>
<td>1·25</td>
<td>2·2</td>
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<tr>
<td>8</td>
<td>1·8/F 5·7</td>
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<td>GH</td>
<td>0·11 (−)</td>
<td>21-OH (2·93)</td>
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<td>NE</td>
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<tr>
<td>9</td>
<td>6·7 F 6·8</td>
<td>0·1</td>
<td>GH</td>
<td>&lt; 0·1 (−)</td>
<td>21-OH (3·02)</td>
<td>4·5</td>
<td>0·3</td>
<td>1·32 (?)</td>
<td>NE</td>
<td>NE</td>
<td>6·4</td>
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<td>−</td>
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<td>1·13 (+)</td>
<td>Neg</td>
<td>4·4</td>
<td>1·8</td>
<td>NE</td>
<td>1·10 ([)</td>
<td>4·4</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>13·1/F 18·2</td>
<td>5·1</td>
<td>GH/FSH-LH</td>
<td>1 : 32</td>
<td>1·25 (+)</td>
<td>Neg</td>
<td>1·9</td>
<td>0·3</td>
<td>NE</td>
<td>&lt; 1 (!]</td>
<td>1·9</td>
<td></td>
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<tr>
<td>12</td>
<td>7·9/F 21·2</td>
<td>13·3</td>
<td>GH/FSH-LH</td>
<td>1 : 8</td>
<td>0·27 (−)</td>
<td>TG (41)</td>
<td>5·8</td>
<td>0·8</td>
<td>1·16 (?)</td>
<td>NE</td>
<td>5·8</td>
<td></td>
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</table>

*Endomysium autoantibodies; parietal cell autoantibodies; glutamic acid decarboxylase autoantibodies; thyroperoxidase autoantibodies; TSH receptor autoantibodies.
IA2 Abs, islet-cell autoantibodies [positive (+) > 1 U/ml]; 21-OH, 21 hydroxylase autoantibodies (positive > 1 U/ml); TG-Abs, thyroglobulin autoantibodies (positive > 40 UI/ml).
†Seroconversion; NE, not evaluated.
‡Patients who were IA2 positive at first determination. 21-OH autoantibodies confirmed positive in cases 8, 9; TG autoantibodies confirmed positive in case 12.

Table 2. Clinical features, pituitary stalk (PS) phenotype, serum AVPc-Abs and organ-specific Abs in CDI-related LCH and germinoma

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age at diagnosis (years)/Sex</th>
<th>Age at time of study (years)</th>
<th>Disease duration (years)</th>
<th>AP hormone deficiencies</th>
<th>AVPc-IgG/IgA (titre)</th>
<th>IA2 Abs (U/ml)</th>
<th>Organ-specific Abs (U/ml)*</th>
<th>PS at first MRI (mm)</th>
<th>Abs follow-up (years)</th>
<th>AVPc-IgG/IgA follow-up (titre)</th>
<th>I2A Abs follow-up (U/ml)</th>
<th>PS at follow-up MRI (mm)</th>
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<td>LCH</td>
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<td></td>
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<tr>
<td>13</td>
<td>3·0/M 8·6</td>
<td>5·6</td>
<td>GH</td>
<td>Neg 1·88 (+)</td>
<td>Neg</td>
<td>1·6</td>
<td>1·3</td>
<td>1·8†</td>
<td>1·0‡</td>
<td>1·05‡</td>
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<tr>
<td>14</td>
<td>0·4/F 21</td>
<td>20·6</td>
<td>GH</td>
<td>Neg/1 : 16</td>
<td>Neg</td>
<td>2·0</td>
<td>0·6</td>
<td>Neg/Neg‡</td>
<td>1·03‡</td>
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<td>15</td>
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<td>GH/FSH-LH</td>
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<td>21-OH (1·23)</td>
<td>6·1</td>
<td>1·2</td>
<td>1·9</td>
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<td>16</td>
<td>7·6/F 15·4</td>
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<td>1·31 (+)</td>
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<td>17</td>
<td>6·6/M 11·1</td>
<td>4·5</td>
<td>GH/TSH</td>
<td>1 : 8</td>
<td>2·0 (+)</td>
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<td>4·3</td>
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<tr>
<td>18</td>
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<td>GH/TSH</td>
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<td>4·9</td>
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<td>NE</td>
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<td>NE</td>
<td>4·9</td>
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<tr>
<td>Germinoma</td>
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<td></td>
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</tr>
<tr>
<td>19</td>
<td>17/M 17·3</td>
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<td>GH/FSH-LH</td>
<td>1 : 64</td>
<td>1·14 (+)</td>
<td>Neg</td>
<td>5·6</td>
<td>0·3</td>
<td>&gt; 1·64 (?)</td>
<td>1·24</td>
<td>2·15†</td>
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<tr>
<td>20</td>
<td>9·4/F 9·6</td>
<td>0·2</td>
<td>GH/ACTH/TSH</td>
<td>Neg/Neg 2·43 (+)</td>
<td>Neg</td>
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<td>0·7</td>
<td>1·16†</td>
<td>1·53</td>
<td>2·45</td>
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</table>

*Endomysium autoantibodies; parietal cell autoantibodies; glutamic acid decarboxylase autoantibodies; thyroperoxidase autoantibodies; TSH receptor autoantibodies.
IA2 Abs, islet-cell autoantibodies [positive (+) > 1 U/ml]; 21-OH, 21 hydroxylase autoantibodies (positive > 1 U/ml); TSH receptor autoantibodies.
†Seroconversion; NE, not evaluated. §After radio-chemotherapy. ¶Patients who were IA2 positive at first determination. 21-OH autoantibodies confirmed positive in case 15; TG autoantibodies confirmed positive in case 12.
were obtained before and after gadopentetate dimeglumine administration (GD-DTPA, 0·1 mmol/kg body weight). A normal pituitary stalk was recorded when the maximum transverse dimension of the stalk was less than 3·0 mm.

**Study protocol**

After a median time-span of 4·9 years from the diagnosis of CDI, all patients underwent the measurement of circulating AVPc-IgG antibodies (AVPc-IgA was available in only six patients) and of the following autoantibodies: antibodies to protein tyrosine phosphatase (IA2), glutamic acid decarboxylase autoantibodies (GAD), 21-hydroxylase autoantibodies (21-OH), endomysium autoantibodies (EMA), parietal cell autoantibodies (PCA), thyroperoxidase autoantibodies (TPOAbs), thyroglobulin autoantibodies (TGAbs) and TSH receptor autoantibodies (TSHrAbs). A second measurement of serum AVPc-IgG levels was performed in 13 patients and of AVPc-IgA in five patients after a median time-span of 0·8 years (range 0·3–1·8 years) following the first evaluation. A personal questionnaire was collected from all patients regarding familial cases of Hashimoto and Grave’s disease, coeliac disease, chronic inflammatory bowel diseases, rheumatoid arthritis, lupus syndrome, diabetes mellitus type 1, Addison disease, vitiligo and myasthenia gravis. AVPcAbs were evaluated in a control group of 40 children (26 females, 14 males) with a mean age of 8·0 ± 2·6 years who were not affected by endocrine diseases.

**Immunological study**

Circulating cytoplasmic AVPc-Abs were identified by using an indirect immunofluorescence method. Unfixed cryostat sections of young normal baboon hypothalamus were initially incubated with the sera. Fluorescein isothiocyanate (FITC)-conjugated goat anti-human immunoglobulins (Igs) and diluted 1:40 sera were used to detect the presence of antibodies to hypothalamic cells. The positive serum samples were subsequently tested with FITC-conjugated goat anti-human IgG and IgA separately. Fresh normal human serum and FITC-conjugated goat anti-human complement factors diluted 1:40 were used to exclude nonspecificity or detect the presence of complement-fixing antibodies. The positive serum samples were tested with specific rabbit anti-AVP serum and rhodamine-conjugated goat anti-rabbit Ig and serum to prove that the antibodies specifically recognized the AVP-secreting cells. Preabsorption of sera by rat liver acetone powder was performed to exclude other organ nonspecific reactivity in detecting all cited antibodies. Two known positive and two known negative sera were chosen for internal controls. The AVPc-Abs were measured in 150 healthy subjects with negative results in all cases. These subjects served as negative controls for the measurement of AVPc-Abs. The levels of AVPc-Abs were considered positive starting at a dilution of 1:2 and were expressed as the endpoint dilution titre; levels below 1:8 were considered at low titre, whereas levels of 1:8 or more were considered at high titre.

The measurement of IA2 Abs was based on a radioligand assay using commercial kits supplied by Medipan GMBH (Selchow, Germany). Serum samples were first incubated with highly purified 125I-labelled recombinant human IA2, followed by the addition of solid-phase protein A to precipitate the labelled IA2–IA2 complexes. For this procedure, after centrifugation, the precipitates are counted for 125I and the amount of radioactivity is proportional to the concentration of IA2 antibody in the test sample. Values were considered normal when the serum IA2 Abs level was less than 1·0 U/ml. Assays to detect autoantibodies to GAD65 (CentAK) were performed by solid-phase radioimmunoassay (RIA) using commercial kits provided by Medipan GMBH; normal values were calculated as a serum GADS level less than 1·25 U/ml. The 21-OH autoantibodies were measured by an immunoprecipitation assay using commercial kits (RSR Ltd, Cardiff, UK). Highly purified 125I-labelled recombinant human 21-OH is first incubated with serum samples, followed by the addition of solid-phase protein A to precipitate the labelled 21-OH–21-OH Ab complexes. After centrifugation, the precipitates are counted for 125I and the amount of radioactivity is proportional to the concentration of 21-OH autoantibody in the test sample. Our laboratory classified normal concentrations as those less than 1·0 U/ml. EMA and PCA were detected by indirect immunofluorescence. Levels of EMA were considered positive starting at 1 : 10 serum dilution in phosphate-buffered saline (PBS) using monkey oesophagus as a substrate and anti-human IgG fluorescence conjugate (INOVA Diagnostic Inc., San Diego, CA, USA); PCA were considered positive starting at 1 : 40 dilution on rat-liver/kidney/stomach with goat anti-human IgG fluorescence conjugate. A BX 51 Olympus fluorescence microscope at 40× power (Olympus Optical Co., Hamburg, Germany) was used. TPO and TG autoantibodies were measured with a solid-phase, enzyme-labelled, chemiluminescent immunometric assay (Immulite 2000 analyser; Medical Systems, Genova, Italy); TPO was considered positive with values of > 10 IU/ml and TG with values of > 40 U/ml. TSHr autoantibodies were measured with THBIA (DiaSorin spa, Vercelli, Italy) by using a two-step radioreceptor assay; TSHr was considered positive with values of > 9 U/l.

**Statistical analysis**

Correlations among the variables were analysed with the Spearman r coefficient. P < 0·05 was considered statistically significant. Comparisons between categorical or nominal variables were analysed with the χ²-test or Fisher’s test (where appropriate). All tests were two-sided. Analyses were performed with Statistica for Windows software (StatSoft, Inc., 2005, Tulsa, OK, USA).

**Results**

The characteristics of the 20 patients are reported in Tables 1 and 2 according to the cause of CDI. All except two patients (one with LCH and one with germinoma) were children at the time of diagnosis and the numbers of male and female patients were similar. Responses to the water deprivation test were compatible with complete CDI in 19 patients and with partial CDI in one. Twelve patients were affected by idiopathic CDI, of whom one (case 12) had a pituitary stalk biopsy compatible with autoimmune-inflammatory disease. In the LCH group, the onset of CDI was concomitant with the diagnosis of LCH in two cases; in three cases polyuria and polydipsia appeared 2·8, 3·8 and 5·7 years after the diagnosis of LCH, respectively. In one case the onset of CDI preceded the diagnosis of LCH by 2·2 years.
In the two patients with germinoma, biopsy-proven diagnosis was obtained 3 and 6 months after the onset of CDI symptoms.

**Anterior pituitary function**

GH deficiency was documented in 16 patients (80%); 10 of these patients (62%) had one or more additional anterior pituitary hormone deficiencies. Five patients had hypogonadotrophic hypogonadism, four patients had ACTH deficiency and three had TSH (Tables 1 and 2). Nine of the 12 patients with idiopathic CDI were affected by GH deficiency, of whom five had one or more additional anterior pituitary hormone deficiencies (Table 1). Only three patients had isolated AVP deficiency. In the LCH group, two patients had GH deficiency and three had MPHD. Both patients with germinoma had MPHD (Table 2).

**Imaging studies**

All patients showed an absence of posterior pituitary hyperintensity at the first MRI evaluation. Nine patients with idiopathic CDI (75%) showed pituitary stalk thickening at the first MRI; four of them had pituitary stalk normalization at the last MRI follow-up, one demonstrated further enlargement of pituitary stalk size and one showed thickening of a previously normal pituitary stalk. Pituitary stalk thickening was documented in three LCH patients (50%) who did not show pituitary stalk size variations during the follow-up. In the two patients with germinoma, we found progressive thickening of the stalk in one case and double brain localization of the disease in the other. The MRI features of the pituitary stalk are reported in Tables 1–2.

**Immunological study**

AVPc-Abs were found at a high titre in 15 patients (75%), either at the first evaluation or during the follow-up. Twelve out of 20 patients (60%) were AVPc-Abs positive at the first immunological evaluation (Tables 1 and 2). In particular, nine patients (75%) with idiopathic CDI had AVPc-Abs at the first evaluation or during follow-up. AVPc-IgG were detected in eight patients with idiopathic CDI (66%) after a median time-span of 4-9 years (range 0-1–13-3 years) from the onset of the disease. Two of the four AVPc-IgG-negative patients were also negative when tested for AVPc-IgA. Among the LCH patients, two were positive for AVPc-IgG after 4-7–7-8 years from the diagnosis of CDI and one was positive for AVPc-IgA. One of the two patients with germinoma was AVPc-IgG positive shortly after diagnosis.

Subsequent to a follow-up time-span ranging from 0-3 to 1-8 years, AVPc-Abs were positive in nine patients among the 13 evaluated subjects (69%) (Tables 1 and 2). Six of these patients had idiopathic CDI, one had LCH and two had germinoma (Tables 1 and 2). Five patients with idiopathic CDI showed a persistence of AVPc-Abs during follow-up, two continued to be constantly negative after a follow-up period of 1-2 and 1-8 years, respectively, and one tested positive at 1-year follow-up (Tables 1 and 2). Four patients showed an increase in AVPc-Abs serum titre during follow-up whereas one showed a decrease in AVPc-Abs titre (Tables 1 and 2). In the three re-evaluated patients with LCH, AVPc-Abs became positive in one, negative in another and one patient remained negative. One patient with germinoma who was previously negative for both AVP-IgG and IgA autoantibodies was found to be AVP-IgA positive 0-7 years later; the other patient showed an increase in AVP-IgG titre at the 3-month follow-up.

Seventeen patients were found to have additional autoantibodies at the time of the first evaluation (Tables 1 and 2). Four patients, with three with idiopathic CDI and one with LCH, had 21-OH or TSH autoantibodies. Fourteen patients (70%) evidenced serum IA2 autoantibodies; specifically, seven of them had idiopathic CDI, five had LCH and two had germinoma. At follow-up, eight patients with autoantibodies remained positive; five of these patients had idiopathic CDI, one had LCH and two had germinoma.

The questionnaire filled out by all of the patients’ parents reported the absence of autoimmune diseases with the exception of two cases of Hashimoto’s thyroiditis in a mother and a sister from the same family of a patient with LCH. None of the 40 control group subjects was positive for AVPc-Abs.

**The relationship between AVPc-Abs, endocrine status and MRI**

AVPc-Abs were present in eight out of nine patients with idiopathic CDI and pituitary stalk thickening either at the time of the first neuroimaging evaluation or at the follow-up. Two out of three patients with idiopathic CDI and normal pituitary stalk had AVPc-Abs. Only one out of the four LCH patients with AVPc-Abs had thickening of the pituitary stalk, whereas the remaining patients had normal pituitary stalk size.

Normalization of the pituitary stalk was observed at the last MRI follow-up in four patients with idiopathic CDI, of whom three evidenced AVPc-Abs consistently. In one patient who showed increased pituitary stalk size, the AVPc-Abs titre increased as well, while a previously normal pituitary stalk became thickened in one patient (case 6) (Fig. 1). Among the LCH patients, MRI follow-up did not demonstrate any significant pituitary stalk size variations. There was no evidence of germinoma lesions at MRI in any of these patients following radio-chemotherapy (Tables 1 and 2).

AVPc-Abs were positive in two out of three (66%) patients with isolated AVP deficiency, in three out of four (75%) patients with AVP and GH deficiency, and in four out of five (80%) patients with AVP and MPHD.

The results of the correlation analyses showed no statistical difference between patients with AVPc-Abs as compared to the other patients, regardless of the aetiology of CDI, the time of antibody evaluation, pituitary stalk morphology or disease duration.

**Discussion**

This study is the first attempt to evaluate the relationship between autoimmunity, pituitary stalk involvement and neuroendocrine function in the youngest ever reported series of patients with CDI and lymphocytic infundibulo-hypophysitis. Our patients differed from those described by Imura *et al.* in that theirs were adult subjects with isolated vasopressin deficiency, self-limiting pituitary stalk and neurohypophysis abnormalities (observed mainly within the first...
Autoimmunity and central diabetes insipidus

2 years from diagnosis) and normal anterior pituitary gland size and function. In addition, our patients were younger at the time of CDI presentation and showed differing spontaneous alterations in pituitary stalk size together with anterior pituitary hormone deficiencies combined with shrinkage of the anterior pituitary gland.

We have shown previously that evolving pituitary stalk lesion and pituitary hormone deficiencies in idiopathic CDI are associated with biopsy-proven lymphocytic infiltration and that pituitary stalk thickening is a feature of autoimmune polyendocrine syndrome associated with CDI.

In the present study, AVPc-Abs were found in 75% of subjects with idiopathic CDI, suggesting that hypothalamic-neurohypophyseal autoimmune involvement is more common in children and young adults with idiopathic CDI than has been generally supposed. Indeed, the frequency of idiopathic vasopressin deficiency recently reported by our group in a large cohort of subjects with CDI (52%) reflects our incomplete knowledge of the causes of this heterogeneous condition. Moreover, more sophisticated noninvasive imaging techniques showed that only 26% of idiopathic CDI subjects who do not have detectable pituitary stalk lesions at MRI might have vascular impairment of the inferior hypophyseal artery system. These findings support the position that the causal mechanism affecting posterior pituitary function remains largely undefined.

The detection of autoantibodies to AVP-secreting cells in three-quarters of our patients with idiopathic CDI who presented during childhood compared to the one-third found in adult patients with identical disease duration underlines the fact that an autoimmune cause of idiopathic CDI is very frequent. Although in the past, idiopathic CDI was ascribed to an autoimmune process involving hypothalamic vasopressin cells in 46% of children, this previous study suffers from the absence of sensitive neuroimaging techniques and from a lack of serum autoantibodies at follow-up. Furthermore, in our study there was no significant female preponderance for AVPc-Abs, the frequency of AVPc-Abs was higher and there was a striking association between AVPc-Abs and thickened pituitary stalk. Although an idiopathically thickened pituitary stalk could be highly suggestive of local lymphocyte infiltration, as well as of potential autoantibody formation, the detection of AVPc-Abs at the time of normalization of a previously thickened pituitary stalk (as in case 5, who was negative for AVPc-Abs at the first evaluation) provides evidence that autoimmunity should not be underestimated either in patients with a thickened pituitary stalk or in subjects with a normal pituitary stalk size. Indeed, the unpredictable behaviour of a pituitary stalk with progressive thickening or spontaneous recovery has been reported as an idiopathic CDI-related feature. The finding of vasopressin-cell autoantibodies in one patient (case 6) with a normal pituitary stalk at first MRI evaluation and subsequent confirmation of increased autoantibodies titre, together with the evolution of the pituitary stalk towards thickening after short-term follow-up, is in favour of possible antibody fluctuation or suggests that AVPc-Abs may even represent a prognostic marker of the first stage of humoral autoimmunity with potential subsequent pituitary stalk involvement by means of lymphocyte infiltration.

AVPc-Abs were found in approximately 77% of our subjects with combined posterior and anterior pituitary dysfunction, a finding that goes well beyond the reported association of anterior pituitary...
hormone defects in as many as 23% of subjects with isolated vasopressin deficiency. This indicates that anterior pituitary involvement in the course of idiopathic CDI is highly suggestive of an autoimmune neurohypophysial basis. The recent discovery that autoantibodies to the median eminence dopaminergic nerve terminals are associated with GH deficiency in APS type 1 may also provide important future insights into the condition of idiopathic CDI. The total absence of AVPc-Abs in healthy children compared to patients with CDI is important because it underlines the specificity of these autoantibodies.

The pattern of AVPc-Abs during the follow-up leaned towards a confirmation of consistently circulating autoantibodies in the majority of the affected subjects, and even the detection of a subsequent AVPc-Abs presence. The demonstration of an increase or a decrease in antibody titre observed in our patients is in agreement with the previously reported pattern of autoantibodies. However, the subsequent identification of AVPc-Abs during the study time-span is in contrast with the position that an absence of AVPc-Abs serves to rule out the subsequent development of these antibodies and a potential consequent autoimmune involvement. In the latter study, the patients with idiopathic CDI and absent circulating AVPc-Abs at the time of disease onset were found to be consistently negative at 5-year follow-up and did not show pituitary stalk thickening over time.

A high frequency of AVPc-Abs was observed in our patients with LCH, a condition characterized by ‘activated’ antigen-presenting cells (histiocytes) in the target-affected tissue. This is in agreement with reported studies in children and adults with LCH and CDI. It is noteworthy that patients with LCH and CDI have radiological and immunological targets similar to those with idiopathic CDI, suggesting that both diseases possibly share a similar pathogenetic mechanism. The pathogenesis of humoral autoimmune activation in the course of LCH has been explained previously by the fact that LCH cells bear class II major histocompatibility antigens on their surface so that specific infiltration of the hypothalamus may trigger T-helper cells to induce an autoimmune reaction to hypothalamic antigens. Moreover, LCH is associated with T-suppressor cell defects that may increase this autoimmune response against hypothalamic cells. Consequently, the presence of AVPc-Abs could be considered an epiphenomenon due to a transient and reversible inflammatory process mediated by lymphocyte migration from the barrier to the hypothalamus favoured by increasing endothelial adhesion to cerebral circulation. Indeed, in our LCH patients the tendency of AVPc-Abs towards clearance or reduction of antibody titre, as compared to idiopathic CDI patients who show the persistent presence of AVPc-Abs or their subsequent appearance in the follow-up, strengthens the hypothesis of a transitory ephiphenomenon in LCH-affected subjects.

Similarly, in idiopathic CDI the possibility that an autoimmune process at the level of the hypothalamic-neurohypophysial system could have been triggered by a viral infection cannot be ruled out, as has recently been proven in a model of a transgenic mouse pituitary that is susceptible to CD8 T-cell-mediated autoimmunity leading to pituitary hypophysitis and dwarfism. In about a quarter of our previously reported patients with idiopathic CDI, there was a temporal association between a viral infection and the onset of polyuria and polydipsia. It seems that such an association between the onset of CDI and varicella infection in eight of our patients (partially published data) cannot simply be considered a chance occurrence, but rather deserves close attention and further study in the future.

The high incidence of AVPc-Abs in the patients with germinoma is not surprising and could even occur in the hypothalamic-median eminence region, where the absence of a blood barrier may open the way for circulating antibodies to reach and potentially affect neuro-endocrine glands. These autoantibodies have not been reported in germinomas before specific treatment, but rather after surgery in craniopharyngioma and in other sellar masses. The production of autoantibodies could be secondary to the host reaction to the tumour (represented by the prominent lymphocyte infiltrations) and to the exposition of autoantigens with consequent stimulation of an autoimmune response. The high specificity of AVPc-Abs, as demonstrated by their absence in normal controls, suggests that evidence of AVPc-Abs in these patients and in those with LCH favours the hypothesis of a secondary phenomenon related to the autoimmune process.

In our patient series with idiopathic CDI, IA2 autoantibodies were the main autoimmune association, although it was not uncommon to observe more than one autoantibody. The combination of multiple autoimmune markers in up to 80% of the cohort and in 83% of the patients with idiopathic CDI without clinical manifestations provides further support for the hypothesis of an autoimmune pathogenesis of idiopathic CDI with involvement of other endocrine glands, organs and tissues. The increased frequency of one specific autoantibody, namely IA2, compared to 21-OH, as well as their combined increased frequency compared to thyroid and other antibodies still needs to be studied and further clarified. Our study differs from others reported in adult populations in terms of the absence of autoimmune diseases in both the patients and their families. Up to one-third of adult CDI patients have been reported to be affected by other endocrine autoimmune diseases. Whether the autoantibodies in our patients could represent an early indicator of a more complex mechanism possibly involving other endocrine glands over time remains to be established. It is recommended that these patients undergo repeated autoantibody evaluations, as well as dynamic stimulation testing (adrenal and endocrine pancreas), because future progression towards clinical diabetes mellitus and/or adrenal insufficiency in subjects with potentially latent autoimmune diseases clearly requires additional study.

In conclusion, the identification of a high frequency of circulating vasopressin-cell autoantibodies in children and young adults with idiopathic CDI has improved our understanding of the aetiology of the most common form of CDI. The autoimmune phenomenon affecting the majority of patients with idiopathic CDI and thickened pituitary stalk cannot be underestimated and may well occur in many other subjects with no detectable pituitary stalk lesions. Our findings demonstrate the utility of measuring AVPc-Abs in children with idiopathic CDI and suggest that the probability of an autoimmune causal mechanism is common during childhood. The identification of AVPc-Abs in subjects who could have either idiopathic CDI or LCH or germinoma, however, indicates that this positive identification cannot be considered a completely reliable marker of autoimmune CDI. Thus, to ensure a definitive aetiological diagnosis of CDI, close clinical and MRI follow-up are needed because AVPc-Abs

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may mask germinoma or LCH. Finally, the present study also underlines the importance of possible further organ involvement in idiopathic CDI patients that could lead to autoimmune diseases in adulthood.

Acknowledgements

We thank Professor R. Moratti of the IRCCS, Policlinico San Matteo, University of Pavia for the routine biochemical analysis of some autoantibodies.

References


ERRATUM

Clinical Endocrinology, 65, 470–478

Idiopathic central diabetes insipidus in children and young adults is commonly associated with vasopressin-cell antibodies and markers of autoimmunity

Mohamad Maghnie, Stefano Ghirardello, Annamaria De Bellis, Natasca di Iorgi, Linda Ambrosini, Andrea Secco, Mara De Amici, Carmine Tinelli, Antonio Bellastella and Renata Lorini

The publisher would like to apologise for the following errors (1), (2) and (3) found in Tables 1 and 2 of the article above:

1. In Table 1, column 10 (Abs follow-up (years)), ‘(–)’ should replace ‘0·3’ for patients 1 and 8.
2. In Table 1, column 11 (AVPc-IgG/IgA follow-up (titre)), ‘(?)’ should be deleted for patients 3, 6, 7, 9 and 12.
3. In Table 2, column 11 (AVPc-IgG/IgA follow-up (titre)), ‘(?)’ should be deleted for patient 19.

The corrected versions of Tables 1 and 2 can be found on the pages overleaf.

Reference

Table 1. Clinical features, pituitary stalk (PS) phenotype, serum AVPc-Abs and organ specific Abs in idiopathic CDI

<table>
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<tr>
<th>Patient no.</th>
<th>Age at diagnosis (yr)/sex</th>
<th>Age at study (yr)</th>
<th>Disease Duration (yr)</th>
<th>AP hormone deficiencies</th>
<th>AVPc-IgG/IgA (titre)</th>
<th>IA2 Abs (U/ml)</th>
<th>Organ specific Abs (U/ml)*</th>
<th>PS at 1st MRI</th>
<th>Abs Follow-up (yr)</th>
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*Endomysium autoantibodies; parietal cell autoantibodies; glutamic acid decarboxylase autoantibodies; thyroperoxidase autoantibodies; TSH receptor autoantibodies.
IA2 Abs, islet-cell autoantibodies [positive (+) > 1 U/ml]; 21-OH, 21 hydroxylase autoantibodies (positive > 1 U/ml); TG-Abs, thyroglobulin autoantibodies (positive > 40 UI/ml).
†Seroconversion; NE: not evaluated.
‡Patients who were IA2 positive at first determination. 21-OH autoantibodies confirmed positive in cases 8, 9; TG autoantibodies confirmed positive in case 12.
**Table 2.** Clinical features, pituitary stalk (PS) phenotype, serum AVPc-Abs and organ specific Abs in CDI-related LCH and germinoma

<table>
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<th>Patient no.</th>
<th>Age at diagnosis (yr)/sex</th>
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*Endomysium autoantibodies; parietal cell autoantibodies; glutamic acid decarboxylase autoantibodies; thyroperoxidase autoantibodies; TSH receptor autoantibodies.
IA2 Abs, islet-cell autoantibodies [positive (+) > 1 U/ml]; 21-OH, 21 hydroxylase autoantibodies (positive > 1 U/ml).
†Seroconversion. ‡Clearance. PS: pituitary stalk; NE, not evaluated. §After radio-chemotherapy. ¶Patients who were IA2 positive at first determination. 21-OH autoantibodies confirmed positive in case 15; TG autoantibodies confirmed positive in case 12.