Longitudinal Study of Vasopressin-Cell Antibodies and of Hypothalamic-Pituitary Region on Magnetic Resonance Imaging in Patients with Autoimmune and Idiopathic Complete Central Diabetes Insipidus


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Diagnosis of autoimmune central diabetes insipidus (CDI) is based on the presence of autoantibodies to AVP-secreting cells (AVPcAb) or the coexistence of other autoimmune polyendocrine syndromes; moreover, it can be also suggested by the presence of lymphocytic infundibulo-neurohypophysitis, evidenced by biopsy of pituitary stalk and/or by pituitary stalk thickening on magnetic resonance imaging (MRI). However, so far, in clinical CDI patients with lymphocytic infundibulo-neurohypophysitis, AVPcAb have not been investigated and in those with or without autoimmune polyendocrine syndromes (APS), longitudinal studies on the behavior of AVPcAb alone, or of both AVPcAb and hypothalamic pituitary imaging on MRI are lacking. Aim of this work was to investigate in these patients the occurrence of AVPcAb (by indirect immunofluorescence) and of pituitary stalk thickening (by MRI) and their longitudinal changes during a follow-up period. We studied 22 patients, aged 29–53, with APS and complete CDI, grouped as follows: 10 with recent onset (<1.5 yr) of CDI (group 1a) and 12 with CDI of long-term duration (≥ 7 yr) (group 1b); moreover, a group of 13 patients with apparent idiopathic CDI of recent onset (<1.5 yr) were studied. They were divided, on the basis of the detection of AVPcAb as follows: 5 AVPcAb positive patients (aged 19–26) classified as isolated autoimmune CDI (group 2) and 8 AVPcAb negative patients (aged 21–26), classified as true idiopathic CDI (group 3). All patients were evaluated yearly, along 5 yr, for AVPcAb and for hypothalamic-pituitary region imaging. At study entry, 8/10 (80%) of patients in group 1a and 7/12 (58.3%) in group 1b were positive for AVPcAb and persisted positive subsequently, during all the follow-up period, even if at lower titers. All patients in group 2 were positive and all those in group 3 were negative for AVPcAb and persisted positive and negative, respectively, for all the follow-up study. Among the AVPcAb-positive patients, only 5 in group 1a and 2 in group 2 showed also pituitary stalk thickening at the first observations, which however spontaneously disappeared subsequently indicating a possible lymphocytic infundibulo-neurohypophysitis. All patients in the studied groups showed loss of the hyperintense signal of the neurohypophysis on MRI at entry and during all the follow-up period. Results of this longitudinal study suggest: 1) AVPcAb, frequently present at high titers in recent phases of CDI, persist subsequently, even if at lower titers, several years after the onset of disease. 2) The occurrence of a lymphocytic infundibulo-neurohypophysitis suggested by the pituitary stalk thickening on MRI only in patients positive for AVPcAb confirms a further autoimmune variant of CDI also in these cases. 3) The longitudinal behavior of patients in group 3 suggests that the absence of AVPcAb at the onset of clinical idiopathic CDI is able to exclude a subsequent appearance of these antibodies and consequently an autoimmune involvement in CDI of these patients. Instead the finding of AVPcAb in several patients with only CDI, thought at first clinical observation as idiopathic, indicates that the prevalence of autoimmune CDI must be considered much higher than that so far reported. (J Clin Endocrinol Metab 87: 3825–3829, 2002)
investigate the presence of AVPcAb and pituitary stalk thickening on MRI; second, to evaluate longitudinally if any change of these findings occurs during a long-term follow-up.

**Subjects and Methods**

On the basis of an AVPcAb screening, performed since 1993 in a large cohort of 2385 patients with APS, 22 patients with complete autoimmune CDI were enrolled in this study. Diagnosis of autoimmune complete CDI was done on the basis of its association with APS and of respective organ specific autoantibodies in presence or absence of AVPcAb. On the basis of duration of autoimmune complete CDI these patients were grouped as follows: group 1a, consisting of 10 patients (8F, 2M, aged 29–36 yr) with recent onset of CDI (≤ 1.5 yr, range 0.5–1.4 yr); group 1b, consisting of 12 patients (9F, 3M, aged 37–53 yr) with CDI of long-term duration (≥ 7 yr, range 7–22 yr). Moreover, 13 patients with apparent idiopathic CDI were also studied; on the basis of the results of AVPcAb they were grouped as follows: group 2, consisting of 5 patients positive for AVPcAb (all females, aged 19–26 yr) thus considered affected by isolated autoimmune CDI, and group 3, consisting of 8 patients negative for AVPcAb (5F, 3M, aged 21–26 yr) considered as true idiopathic complete CDI also because a familial or secondary CDI was excluded.

All patients were under desmopressin-acetate treatment. At the first observation (off treatment) the diagnosis of CDI had been done on the basis of clinical and laboratory findings according to the accepted criteria (5). In particular, in all patients of all groups studied, after fluid deprivation, urine and plasma osmolality were less than 300 and 300 mOsm/kg, respectively. All patients gave their informed consent to the study, which was approved by local ethical committee.

Follow-up study

In all patients AVPcAb and hypothalamic-pituitary region on MRI were evaluated yearly for 5 yr.

Autoantibodies to AVPcAb

AVPcAb were detected by immunofluorescence method on cryostat sections of young baboon hypothalamus, as described previously (11, 12). In particular, fluorescein isothiocyanate-conjugated goat antihuman IgG sera were used to detect the presence of antibodies to hypothalamic cells, and then positive serum samples were tested with fluorescein isothiocyanate goat antihuman IgG, IgM and IgA sera separately. The specificity of the reaction to vasopressin cells was demonstrated with a four-layer double fluorochrome immunofluorescence test in which the second sandwich consisted of rabbit antivasopressin and antioxytocine sera reacting with rabbit-dilaminated goat antirabbit IgG (levels of AVPcAb, considered positive starting at dilution 1/2, were expressed as end-point dilution titer.

One hundred healthy subjects matched for sex and years were used as negative control for AVPcAb.

**MRI studies**

An MRI of hypothalamic-pituitary region was performed by a 0.5 T Vectra scanner (General Electric, Milwaukee, WI) using T1 weighted gradient echo acquisitions (repetition time, 250 ms; flip angle, 90 degree, four signal averages) in the sagittal and coronal planes. In each measurement, we obtained seven slices centered on the posterior pituitary and stalk region. The slices were 3 mm thick with an in-plane spatial resolution of 0.94 mm (180 x 240 mm² field of view, 192 x 256 matrix in the sagittal acquisitions, 150 x 180 mm² field of view, 160 x 192 matrix in the coronal acquisitions). These acquisitions were repeated before and after the administration of 0.1 mm/kg of body weight of gadolinium diethylenetriamine pentacetate analyzing the perfusion with a temporal resolution of 57 sec.

The evaluation of the MRI was performed twice by one operator (F.D.S.) blind in respect to the etiology of CDI of patients of the current study. MRI evaluation included the detection of neurohypophyseal bright spot (presence or absence) and of transverse dimension of pituitary stalk (17). We considered the upper limit of normal maximum transverse stalk dimension of 3.5 mm. according to Simmons et al. (17) with some modifications on the basis of our control group data. Pituitary stalk thickening was graded as minimally thickened (from 3.4–4.5 mm), moderately thickened (from 4.6–6.5 mm) or severely thickened (more than 6.5 mm) according to Maghnie et al. (18) with minor modifications.

**Statistical analysis**

Statistical significance of AVPcAb titer and pituitary stalk thickening variations in antibody-positive patient groups at all times of observation were calculated by ANOVA. AVPcAb titers in all patient groups at all times of observation were correlated to the presence of pituitary stalk thickening by the Spearman’s test. In all tests, a P value less than 0.05 was considered significant.

**Results**

None of patients in all groups showed impairment of pituitary hormonal function, but all of them showed loss of the posterior pituitary hyperintense signal on MRI during all the follow-up periods (data not shown).

Variations of AVPcAb titers and of MRI scans of pituitary stalk during the follow-up period were summarized for group 1 (1a and 1b) in Table 1 and for groups 2 and 3 in Table 2, respectively.

**AVPcAb behavior**

AVPcAb (all of class IgG) were found in 8 of 10 patients (80%) in group 1a and in 7 of 12 patients (58.3%) in group 1b, with titers ranging from 1/32 to 1/8 and from 1/8 to 1/2, respectively (Table 1). Subsequently, during the follow-up period, AVPcAb were persistently present in all previously AVPcAb-positive patients of both groups, even if at lower titers ranging finally from 1/8 to 1/4 in group 1a and from 1/4 to 1/2 in group 1b, respectively (Table 1); P value was less than 0.008 with respect to the starting titers only in group 1a (not shown in table). All patients in group 2, initially AVPcAb positive at study entry, with titers ranging from 1/32 to 1/8, persisted positive for all the follow-up even if
at lower titers (Table 2); P value was less than 0.01 with respect to the starting titers (not shown in table). Two of them during the follow-up became positive for thyroglobin antibodies and thyroperoxidase antibodies developing subsequently an autoimmune thyroiditis. All AVPcAb-negative patients at study entry in groups 1a, 1b (Table 1), and in group 3 (Table 2) persisted constantly negative at later subsequent observations. None of them showed other organ-specific antibodies or autoimmune endocrine diseases.

**MRI findings**

As shown in Table 1, in group 1a pituitary stalk thickening was observed in 3 of the 8 AVPcAb-positive patients at start of the study and in other 2 patients 1 yr after the first MRI observation. In particular, in all 5 patients total stalk was involved, pituitary stalk was minimally or moderately thickened especially after the administration of gadolinium. Subsequently, pituitary stalk thickening markedly improved over time in all patients during the follow-up with a signif-

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### TABLE 1. Behavior of AVPcAb and PS during the follow-up in group 1a and group 1b patients with autoimmune complete CDI

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<td>PS (mm)</td>
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**TABLE 2. Behavior of AVPcAb and PS during the follow-up in group 2 patients with autoimmune isolated complete CDI and in group 3 with idiopathic complete CDI**

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<td>PS (mm)</td>
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**PS, Pituitary stalk (upper limit of normal maximum transverse stalk dimension: 3.3 mm). The numbers in bold indicate patients with a significant pituitary stalk thickening.**

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*At this observation, these patients showed appearance of TgAb and TPOAb, developing subsequently an Hashimoto’s thyroiditis.*
icantly complete reversal at the end of the study (Table 1). $P$ value was less than 0.006 with respect to the starting values (not shown in table).

In group 2, one patient at start of study, and another, 1 yr after the first observation, showed pituitary stalk thickening which subsequently decreased progressively until normalization at the last observation (Table 2). None of patients in group 1b (Table 1) and in group 3 (Table 2) showed pituitary stalk thickening during the study span. A positive correlation between AVPcAb titers and pituitary stalk thickening was found in group 1a initially ($r = 0.89; P = 0.000$) and at all times of observation until to end of the study ($r = 0.86; P = 0.01$) and in group 2 only at the first observation ($r = 0.91; P = 0.02$).

Discussion

Sherbaum et al. (10) showed that some cases of CDI in adult people may be part of APS, in which well established autoimmune diseases may be associated clinically or serologically with CDI, suggesting the concept of an autoimmune pathogenesis of this disease in a subgroup with defined pathological findings. Patients with APS are a good source for the detection of various organ-specific autoantibodies as islet cell antibodies and glutamic acid decarboxylase antibodies in type 1 diabetes mellitus, adrenal cortex antibodies and 21-hydroxylase antibodies in autoimmune Addison's disease (14, 15), and also AVPcAb in autoimmune CDI (19, 20). In a previous study, it has been shown in a single patient with autoimmune CDI a decrease in titers of AVPcAb during the follow-up, suggesting that these antibodies may disappear over the years, indicating the necessity of longitudinal studies to clarify this question (10). So far, longitudinal studies about changes of AVPcAb levels over time in the literature are lacking, except for our study on the longitudinal behavior of AVPcAb in subclinical autoimmune CDI patients (12). In the present study, we investigated the time course of AVPcAb in patients with clinical autoimmune CDI with and without APS and in those with idiopathic complete CDI. In fact, even if these antibodies do not play a pathogenetic role in autoimmune CDI, so far they can be considered a good serological marker of autoimmune CDI (12). Our study demonstrated that AVPcAb persist over time during the follow-up period in previous AVPcAb positive patients with or without other autoimmune diseases, including those with a long duration of disease. This persistence, in the absence of functional activity of target cells, could depend on the presence of residual cells unable to function, but still able to present antigens to the immune system.

Another important point of this study is the longitudinal behavior of patients in group 3, suggesting that the absence of AVPcAb at the onset of clinical idiopathic CDI is able to exclude a subsequent appearance of these antibodies and consequently an autoimmune involvement in CDI of these patients.

It has been observed that lymphocytic infundibulo-neurohypophysitis in CDI patients can be evidenced by a pituitary stalk biopsy able to reveal a lymphocytic plasma-cell infiltration, or suggested by MRI scans able to reveal a pituitary stalk thickening, which can disappear over time spontaneously or after corticosteroid therapy (6, 21–24). However, pituitary stalk biopsy is usually reserved to patients with severe and progressive thickening, but not to those with minimal or moderate thickening, as occurring in our patients (18, 25). The presence of lymphocytic infundibulo-neurohypophysitis in these patients can be suggested by the presence of both pituitary stalk thickening and AVPcAb with or without other subclinical and clinical autoimmune diseases. Pituitary stalk thickening was observed in some autoimmune complete CDI patients with short duration of disease but not in those with long duration of disease or in those with idiopathic CDI. Interestingly, pituitary stalk thickening was present in AVPcAb-positive but not in AVPcAb-negative patients, indicating a strong relationship between the occurrence of these antibodies, markers of autoimmune hypothalamic involvement, and the lymphocytic infundibulo-neurohypophysitis. This is in favor of a further autoimmune variant of CDI also in these cases, even if the lack of histological findings suggests caution against this assumption. However, these results, together with the finding of AVPcAb in several patients with only CDI, thought at first clinical observation as idiopathic, indicates that the prevalence of autoimmune CDI must be considered much higher than that so far reported.

Acknowledgments

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A.D.B. and A.C. have contributed equally to the manuscript.

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