Antipituitary Antibodies in Adults with Apparently Idiopathic Growth Hormone Deficiency and in Adults with Autoimmune Endocrine Diseases

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The role of antipituitary antibodies (APA) in autoimmune pituitary diseases still needs to be clarified. The aim of this study was 2-fold: first, to investigate the presence of APA in adults with idiopathic or acquired GH deficiency (GHD) and in adults with autoimmune endocrine diseases; and second, to evaluate whether in autoimmune endocrine patients APA titer is correlated to the pituitary function and particularly to GH secretion. We studied 12 adults with isolated and apparently idiopathic GHD who were treated with recombinant GH in childhood (group 1a), 14 patients with adult GHD secondary to surgery for pituitary and parasellar tumors (group 1b), and 180 patients with organ-specific autoimmune diseases (group 2). APA were evaluated by indirect immunofluorescence. In all APA-positive patients and in 20 APA-negative patients of group 2, GH secretion was investigated by testing its response to insulin-induced hypoglycemia (insulin tolerance test) and, when impaired, also to arginine.

APA were found (at high titers) in 4 of 12 patients of group 1a (33.3%) but were absent in all patients in group 1b. APA were also found in 40 of 180 patients of group 2 (22.2%), 35 of them at low titers (group 2a) and 5 at high titers (group 2b).

Twenty of the 140 autoimmune endocrine APA-negative patients studied (group 2c) and all APA-positive patients at low titers (group 2a) had normal pituitary function. Conversely, all APA-positive patients at high titers (groups 1a and 2b) had a severe isolated GHD. An inverse correlation between APA titers and GH peak serum response to insulin tolerance test in autoimmune endocrine patients was observed.

Our results suggest that APA, when detected at high titers, may be considered a good diagnostic tool to highlight the possible occurrence of GHD in adults with autoimmune endocrine diseases. Moreover, they may indicate an autoimmune pituitary involvement in adults with apparently idiopathic GHD, suggesting that the prevalence of autoimmune GHD is much higher than that so far considered. (J Clin Endocrinol Metab 88: 650–654, 2003)

Goudie and Pinkerton (1) described the first case of lymphocytic hypophysitis in 1967 and proposed that autoimmunity could play a role in affecting the pituitary gland. Subsequently, the occurrence of an autoimmune hypophysitis was confirmed by the findings of lymphocytes and plasma-cell infiltration on autopsy/biopsy of the pituitary gland (2, 3). Lymphocytic hypophysitis could be suggested also by an enlargement of the pituitary on magnetic resonance imaging (MRI; Refs. 4 and 5). Moreover, proven or suspected lymphocytic hypophysitis is frequently associated with other autoimmune endocrine diseases (6–8). In the majority of cases, autoimmune hypophysitis determines isolated partial or total hypopituitarism due to loss of selective adenohypophysial cells or to more diffuse pituitary damage, respectively (7, 8). In particular, isolated or combined ACTH, TSH, or LH/FSH deficiency has been so far described (9–11). Autoimmune pituitary involvement could be suspected by the presence of antipituitary antibodies (APA; Ref. 7). However, although other organ-specific antibodies are considered good markers of the respective endocrine diseases (12–14), APA, because of several methodological problems, are not considered very specific and sensitive markers of autoimmune pituitary disease (15, 16). APA have been detected not only in some patients with lymphocytic hypophysitis and pituitary hormone deficiency but also in patients with pituitary adenomas, primary empty sella syndrome, and autoimmune endocrine diseases but without pituitary function impairment (8, 17–21). Moreover, APA have been detected frequently in patients with isolated ACTH deficiency and in some patients with isolated LH/FSH or TSH deficiency (22–24). Little data have been reported in the literature on APA in patients with GH deficiency (GHD; Refs. 16, 21, 25, and 26).

The aim of this study was 2-fold: first, to investigate the presence of APA in adults with idiopathic isolated severe GHD, diagnosed in childhood and confirmed in adulthood, in adults with acquired post-pituitary surgery GHD and in adults with autoimmune endocrine diseases; and second, to evaluate whether in autoimmune endocrine patients APA titer is correlated to the pituitary functional state and particularly to GH secretion.

Patients and Methods

We studied 26 patients with adult GHD and 180 patients with autoimmune endocrine diseases.

Twelve of 26 patients (group 1a, 7 females and 5 males; aged 23–27 yr) with adult GHD had a past history of well known childhood-onset GHD without organic hypothalamic-pituitary disorders. All of them had...
been treated with recombinant GH in childhood until skeletal growth was completed, and then this therapy was stopped in all patients at least 5 yr before the revaluation of GH secretion in adulthood. All patients of this group had a good growth response during childhood after GH therapy, without significant differences among them.

The remaining 14 of 26 patients (group 1b, 5 females and 9 males; aged 31–50 yr) had an adult GHD secondary to surgery for pituitary and parasellar tumors performed at least 6 yr before. In particular, all of them had had transsphenoidal and/or transcranial surgery route: eight for nonsecreting pituitary adenomas and six for craniopharyngiomas. Subsequently, four of them developed panhypopituitarism and central diabetes insipidus (CDI), three developed panhypopituitarism alone, four developed FSH/LH and TSH deficiency, and three developed FSH/LH and ACTH deficiency. Hormone replacement with t-thyroxine, cortisone acetate, and intranasal desmopressin had been given appropriately. Hypogonadism had been treated with testosterone enanthate in men and a standard combined estrogen-progesterone preparation in women.

Diagnosis of adult GHD was formulated in all patients according to the recommendation of the Growth Hormone Research Society (27). In particular, the response to insulin-induced hypoglycemia [insulin tolerance test (ITT)] and to arginine was considered impaired with a GH peak of less than 3 μg/liter and less than 1.0 μg/liter, respectively, as previously described (28, 29) with our minor modifications. According to these criteria, all patients in groups 1a and 1b presented with severe untreated GHD. Moreover, they had serum IGF-I levels below the normal range for age, sex, and nutritional state. GHD was considered as isolated when normal basal and dynamic secretion of other pituitary hormones was observed. None of the adults with childhood-onset GHD had a past history of cranial traumas or hypothalamic pituitary abnormalities on MRI. They were thus considered as being apparently idiopathic GHD, on the basis of these findings.

Moreover, 180 patients (group 2, 129 females and 51 males; aged 29–50 yr) with autoimmune endocrine diseases [80 with Hashimoto’s thyroiditis, 59 with Graves’ disease, 15 with Addison’s disease, 17 with latent diabetes in adults (LADA), 9 with CDI] were also studied. Patients with hypothyroid Hashimoto’s thyroiditis, Addison’s disease, and CDI received appropriate replacement therapy. Hyperthyroid Graves’ patients and LADA patients received antithyroid and oral hypoglycemic drugs, respectively. The patients with hypothyroid Hashimoto’s thyroiditis were euthyroid under T4 replacement therapy for at least 5 yr. Hormonal pituitary function was also appropriately investigated in all patients of group 2. In particular, basal anterior pituitary hormones were evaluated in duplicate; ACTH, GH, TSH, FSH, LH, and prolactin (PRL) were determined by immunoradiometric analysis method using commercial kits. Dynamic behavior of these hormones was also studied by testing their response to CRH, TRH, and GnRH. Additionally, target organ hormones (free T4, free T3, testoster-
one, estradiol, progesterone, and cortisol) and IGF-I were also evaluated using commercial kits. As regards group 2, in all APA-positive patients and in a group of APA-negative patients matched for age, sex, and body mass index (BMI) to positive patients, GH was also investigated by testing its response to ITT and, when impaired, also to arginine. We chose these stimuli and not others suggested by some important reports (30, 31), taking into account the cost-benefit ratio, but also because none of our patients had diseases contraindicating the ITT (in fact patients with LADA were not tested, being all APA negative; see Results). Furthermore, IGF-I levels were also evaluated in these patients. Finally, an MRI of the hypothalamic and pituitary region was performed in all of these patients.

All subjects gave their informed consent to the study, which was approved by the local ethical committee.

**APA**

APA were evaluated in all patients in groups 1a, 1b, and 2. Sera from 20 patients (11 males and 9 females; aged 29–52 yr) with pituitary adenomas (12 with acromegaly, 4 with PRL-secreting adenoma, and 4 with nonsecreting adenoma) and sera from 50 normal subjects matched for sex and age were also used as controls for detecting APA.

APA were detected by immunofluorescence method as described previously, with minor modifications (12, 13, 32) on cryostat sections of young baboon pituitary gland. In particular, fluorescein isothiocyanate-conjugated goat antihuman Ig sera were used to detect the presence of APA, and then positive serum samples were tested with fluorescein isothiocyanate goat antihuman IgG, IgM, and IgA sera separately. All sera were tested blindly, and two investigators evaluated the results in a double-blind manner. The samples were considered APA positive when showing an immunofluorescence pattern with strong granular intracytoplasmatic staining in some pituitary cells (about five in each field), but not a diffuse intracytoplasmatic staining involving the majority of the pituitary cells (21). Levels of APA were considered positive starting at dilution 1:2 and were expressed as end-point dilution titer. Finally, only in patients with apparently idiopathic GHD previously treated with human recombinant GH, the sera positive for APA were preabsorbed with an excess of GH (10–15 μg) for 18 h at 4°C and then retested to exclude a possible presence of antibodies against GH.

**Statistical analysis**

Statistical analysis in APA-positive and APA-negative patients in group 2 was performed by ANOVA. The difference between frequencies of APA-positive patients with respect to APA-negative patients in group 2 was calculated by χ2 test. The correlation between APA titers in group 2 and GH peak concentration after ITT was examined by the Spearman’s rank-correlation analysis. In all tests, a P value less than 0.05 was considered significant.

**Results**

The behavior of APA in patients with GHD (groups 1a and 1b), patients with autoimmune endocrine diseases (group 2), those with pituitary adenomas, and normal controls is depicted in Fig. 1.

APA were found in 4 of 12 patients of group 1a (33.3%) with titers ranging from 1/32 to 1/64. They were all of class IgG (immunoreactivity to antihuman IgG but not IgM and IgA sera). Immunofluorescence pattern was characterized by a granular intracytoplasmatic staining in some pituitary cells but not by diffuse intracytoplasmatic staining involving the majority of the pituitary cells. No diminution of immunostaining was observed when sera of these patients were preabsorbed with GH. There were no differences in clinical and hormonal findings between the four patients with isolated childhood-onset GHD with high titers of APA and the remaining eight patients of this group with undetectable APA; however, APA were absent in all patients in group 1b. With respect to the 180 patients in group 2, 40 of them (22.2%; 29 females and 11 males; aged 30–40 yr) were APA positive (of them 23 had Hashimoto’s thyroiditis, 12 had Graves’ disease, 3 had CDI, and 2 had Addison’s disease). In particular, 35 of 40 APA-positive autoimmune patients (87.5%) had APA at low titers ranging from 1/2 to 1/8 (group 2a), whereas 5 of 40 (12.5%) had APA at high titers ranging from 1/32 to 1/64 (group 2b), and the remaining 140 were APA negative (group 2c). APA were also found in 6 of 20 patients (30%) with pituitary adenomas (4 with acromegaly and 2 with PRL-secreting adenoma) and in 2 of 50 (4%) normal controls, but always at low titers (from 1/2 to 1/8). All autoimmune endocrine APA-positive patients at low titers (<1/8) and all APA-negative patients had a normal pituitary function, including GH secretion, whereas all APA-positive patients at high titers (>1/8) had a severe GHD. The characteristics of autoimmune endocrine patients in group 2a, in group 2b, and in 20 of 140 patients of group 2c (in whom ITT was also performed) matched for sex, age, and BMI to patients of groups 2a and 2b are summarized in Table 1. Sex, age, and BMI were not significantly different in all groups of auto-

**Table 1.** Characteristics of autoimmune endocrine patients in groups 2a, 2b, and 2c.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>BMI (kg/m²)</th>
<th>GH secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>9</td>
<td>30–40</td>
<td>19.5–25.5</td>
<td>normal</td>
</tr>
<tr>
<td>2b</td>
<td>11</td>
<td>30–40</td>
<td>19.5–25.5</td>
<td>low</td>
</tr>
<tr>
<td>2c</td>
<td>140</td>
<td>30–40</td>
<td>19.5–25.5</td>
<td>normal</td>
</tr>
</tbody>
</table>

GH secretion: normal, <1/8; low, >1/8. BMI was not significantly different in all groups.
immune endocrine patients. However, GH peak after ITT and IGF-I values in group 2b patients were significantly lower than those observed in group 2a and in 20 patients of group 2c (*P* < 0.001 vs. both groups). Individual parameters of patients of group 2b are illustrated in Table 2. All patients showed serum GH peak less than 3 g/liter after ITT and less than 1.0 g/liter after arginine and low levels of IGF-I, but none of them showed impairment of other pituitary hormone secretion. Finally, APA titers were inversely correlated to GH peak serum responses to ITT in autoimmune endocrine patients (*r* = −0.36; *P* < 0.03). With regard to the characteristics of the MRI of the hypothalamus and sellar region, all patients in group 2a and the 20 of 140 of group 2c, in whom the study was performed, showed normal morphological imaging. Among patients in group 2b, three showed normal imaging, one had MRI characteristics suggestive of lymphocytic adenohypophysitis (enlarged pituitary gland and on dynamic MRI a delay in pituitary enhancement; Ref. 5) and probable lymphocytic infundibulo-neurohypophysitis (pituitary stalk thickening and absence of neurohypophyseal signal; Ref. 33), and another had partial empty sella.

### Discussion

Childhood-onset GH deficiency, subsequently reconfirmed in adulthood, can be caused by genetic abnormalities of hypothalamus or pituitary hormone synthesis or secretion, or may be secondary to destructive hypothalamus or pituitary lesions (trauma, tumors, infiltrative diseases, surgery, irradiations, and more rarely to lymphocytic hypophysitis) that occurred in childhood (34). In the absence of all the above mentioned etiological factors, GHD is usually classified as idiopathic. Circulating APA and, particularly, autoantibodies that selectively stained GH-producing cells have been detected by the immunofluorescence method in rare cases of apparently idiopathic GHD but also in some normal subjects (16, 21, 25). On the other hand, Crock et al. (26), using an immunoblotting method, demonstrated APA in only a small population of patients with idiopathic GHD but not in normal subjects. This suggests that this method could be more specific with respect to conventional immunofluorescence assay for detecting autoimmunity to the pituitary. The nature and the clinical significance of APA are still discussed. The recent identification of the 49-kDa autoantigen associated with lymphocytic hypophysitis as α-enolase suggests that autoantibodies to α-enolase could be good markers of autoimmune

### Table 1. The characteristics of APA-positive patients (groups 2a and 2b) and 20 of 140 APA-negative patients with autoimmune endocrine diseases (group 2c)

<table>
<thead>
<tr>
<th>Group 2a (n = 35)</th>
<th>Group 2b (n = 5)</th>
<th>Group 2c (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (F/M)</strong></td>
<td>22/13</td>
<td>4/1</td>
</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td>38.5 ± 5.5</td>
<td>34.8 ± 3.7</td>
</tr>
<tr>
<td><strong>APA titer (range)</strong></td>
<td>1/2–1/8</td>
<td>1/34–1/64</td>
</tr>
<tr>
<td><strong>HT (n)</strong></td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td><strong>Graves’ disease (n)</strong></td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td><strong>AD (n)</strong></td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><strong>CDI (n)</strong></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>24.6 ± 2.7</td>
<td>25.6 ± 2.3</td>
</tr>
<tr>
<td><strong>Peak GH response to ITT (μg/liter)</strong></td>
<td>12 ± 3.7</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td><strong>IGF-I (μg/liter)</strong></td>
<td>203.1 ± 23.1</td>
<td>96.2 ± 16.7</td>
</tr>
</tbody>
</table>

HT, Hashimoto’s thyroiditis; AD, Addison’s disease; F, females; M, males.
pituitary disease (35). From this point of view, α-enolase can be considered one of the targets of APA, such as 21-hydroxylase in autoimmune Addison’s disease can be identified as one of targets of adrenocortical autoantibodies (36). Concerning the clinical significance, some APA are harmless, whereas others can activate intracellular signaling and exert biological function.

In the present study, we investigated serum APA by immunofluorescence in adults with childhood-onset GHD and in those with GHD secondary to surgery for pituitary and parasellar tumors. The first important result emerging from this study is that APA are present (at high titers) in 4 of 12 (33.3%) patients with idiopathic GHD. Our results suggest that some (1 of 3) patients, previously labeled as idiopathic GHD, seem to have an autoimmune involvement of the pituitary gland.

In our study, we detected APA using immunofluorescence assay and not methods thought to be more specific to confirm whether the immunostained pituitary cells are GH-secreting cells. However, the lack of variations of immunostaining when APA-positive sera were preabsorbed with GH seems to exclude the possibility that the APA are antibodies against GH that were raised during previous recombinant GH treatment.

On the contrary, we showed an absence of APA in all adult patients with GHD secondary to surgery for pituitary and parasellar tumors. However, Crock (8) demonstrated by immunoblotting method high titers of APA not only in patients with lymphocytic hypophysitis but also in one patient submitted to pituitary surgery. This apparent discrepancy with our results probably depends on the different time of APA detection from the time of the surgery rather than by the different method used. Concerning this, a longitudinal study of APA in patients from surgery onward could be useful and is currently in progress.

We investigated APA also in patients with autoimmune endocrine diseases, with pituitary adenomas, and in normal controls. According to previous studies (8), APA were found in autoimmune endocrine patients (22%), in those with pituitary adenomas (30%), and in normal controls (4%). However, all APA-positive patients with pituitary adenomas and APA-positive normal controls have low titers of these antibodies (cut-off limit ≤1/8) suggesting that they could be considered only an epiphenomenon in these cases.

Bottazzo et al. (20), in screening APA by immunofluorescence in sera of a cohort of patients with autoimmune polyglandular diseases, found some of them positive for APA at low titers but without evidence of pituitary function impairment. This seems to support the hypothesis that APA cannot be considered autoimmune markers of pituitary insufficiency. Our results are in agreement with this assumption, but only with respect to the presence of APA at low titers. In fact, all APA-positive autoimmune endocrine patients with low titers (≤1/8) showed normal pituitary function and normal morphological imaging of the pituitary gland, whereas all APA-positive patients with high titers (>1/8) showed an isolated severe GHD and normal imaging (in three patients) or imaging suggestive of lymphocytic adenohypophysitis and lymphocytic infundibulo-neurohypophysitis and/or partial empty sella (in the other two cases, respectively). Thus, we conclude that APA, only when present at high titers, can be considered as good autoimmune markers of pituitary impairment in autoimmune endocrine

### Table 2. The characteristics of the autoimmune endocrine patients with presence of APA at high titers (group 2b)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>BMI (kg/m²)</th>
<th>Autoimmune endocrine disease</th>
<th>Organ-specific antibodies</th>
<th>Peak GH of ITT (ng/liter)</th>
<th>Peak GH of arginine (ng/liter)</th>
<th>IGF-I (ng/liter)</th>
<th>Function of other anterior pituitary hormones</th>
<th>MRI of pituitary and hypothalamus</th>
<th>APA titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>F</td>
<td>27</td>
<td>HT</td>
<td>TgAb, TPOAb</td>
<td>23</td>
<td>0.8</td>
<td>108</td>
<td>Normal</td>
<td>Normal</td>
<td>1/32</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>F</td>
<td>25</td>
<td>HT</td>
<td>TgAb, TPOAb</td>
<td>2</td>
<td>0.6</td>
<td>99</td>
<td>Normal</td>
<td>Normal</td>
<td>1/64</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>F</td>
<td>26</td>
<td>HT</td>
<td>TgAb, TPOAb</td>
<td>1.7</td>
<td>0.2</td>
<td>77</td>
<td>Normal</td>
<td>Normal</td>
<td>1/32</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>F</td>
<td>22</td>
<td>CDI</td>
<td>AVPcAb</td>
<td>1.9</td>
<td>0.5</td>
<td>85</td>
<td>LH/LINH</td>
<td>Partial empty sella</td>
<td>1/64</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>M</td>
<td>28</td>
<td>HT</td>
<td>TPOAb</td>
<td>2.5</td>
<td>0.4</td>
<td>104</td>
<td>Normal</td>
<td>Normal</td>
<td>1/64</td>
</tr>
</tbody>
</table>

TgAb, Thyroglobulin antibodies; TPOAb, thyroperoxidase antibodies; AVPcAb, vasopressin cell antibodies; HT, Hashimoto’s thyroiditis; LH, lymphocytic hypophysitis; LINH, lymphocytic infundibulo-neurohypophysitis.
patients. Moreover, the finding of APA at high titers in some patients with apparently idiopathic GHD and in patients with GHD associated to autoimmune endocrine diseases in the absence of alteration in the secretion of other pituitary hormones, as well as the inverse correlation between APA titers and the serum peak GH response to ITT, suggest an autoimmune mechanism as the cause of GH impairment in these cases. This also indicates that somatotrophs can be an early target of the autoimmune process. On the other hand, the absence of APA in patients with GHG secondary to surgery for pituitary and parasellar tumors seems to exclude that APA are the result of pituitary gland damage. In conclusion, our results suggest that APA could be considered a good diagnostic tool for highlighting the possible occurrence of a form of GHD, thus inducing the performance of dynamic tests for GH secretion in patients with autoimmune endocrine diseases positive for APA at high titers. Moreover, they may indicate an autoimmune pituitary involvement in adults with apparently idiopathic GHD, suggesting that the prevalence of autoimmune GHD is much higher than previously considered.

Acknowledgments

Received July 8, 2002. Accepted November 14, 2002.

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This work was supported in part by grants from Ministero Università e Ricerca Scientifica e Tecnologica (PRIN 2001.06439-003 to A.B.). A.D.B. and A.B. contributed equally to the manuscript.

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