

ORIGINAL ARTICLE

Characterization of antipituitary antibodies targeting pituitary hormone-secreting cells in idiopathic growth hormone deficiency and autoimmune endocrine diseases

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Summary

Objective In order to investigate whether somatotrophs are the target of antipituitary antibodies (APA) in adult patients with growth hormone deficiency (GHD), we studied the sera of 37 APA positive patients.

Patients Patients were grouped as follows: nine patients with APA at high titre (> 1 : 8) affected by apparently idiopathic GHD; four of them (group 1a) with isolated GHD diagnosed during childhood and five with GHD diagnosed during adulthood associated with autoimmune endocrine diseases (group 1b), and 28 patients with autoimmune endocrine diseases without pituitary impairment, previously found positive for APA at low titre (1 : 8, group 2).

Measurements APA were evaluated by a four-layer double indirect immunofluorescence technique.

Results In group 1a patients, APA immunostained exclusively GH-producing cells. In group 1b patients, APA were directed not only to GH- but also to other pituitary hormone-producing cells. In group 2 patients, APA were directed selectively to PRL-producing cells and rarely to some GH-producing cells.

Conclusions In the present study, we demonstrated that GH-secreting cells are the target of the autoimmune reaction in autoimmune GHD and that the immunostaining of only the somatotrophs is typical of isolated GHD. In contrast, the finding of diffuse staining of APA indicates the need to search for other autoimmune diseases. Finally, the presence of APA at low titre directed against PRL-secreting cells in patients with autoimmune endocrine diseases in the absence of pituitary impairment, seems to be only a nonspecific marker of pituitary autoimmunity. A longitudinal study would be useful to clarify the relationship between the different pituitary cell involvement

and the natural history of pituitary dysfunction in autoimmune hypophysitis.

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Many diseases previously considered as idiopathic have long been recognized as autoimmune.^{1–6} In particular, in apparently idiopathic hypopituitarism, the finding of anterior pituitary lymphocytic infiltration by biopsy indicates an autoimmune pathogenesis.^{7–11} Moreover, antipituitary antibodies (APA) are present not only in many patients with hypophysitis diagnosed by biopsy, but also in some patients with idiopathic hypopituitarism with or without autoimmune diseases.^{12–16} This suggests that these antibodies may be a useful marker of autoimmunity when pituitary biopsy cannot be performed. Several years ago, APA were found in 1/4 patients with idiopathic growth hormone deficiency (GHD) by immunofluorescence¹⁷ and in a small population of these patients by immunoblotting method.^{18,19} Subsequently, the target of these antibodies has been identified as a 49 kD pituitary cytosolic protein corresponding to α -enolase.^{19,20} Recently, we searched for APA by indirect immunofluorescence technique in adult patients with idiopathic and secondary GHD and in patients with autoimmune endocrine diseases.²¹ We found that in some patients with apparently idiopathic GHD, either isolated or associated with autoimmune endocrine diseases, the presence of APA at high titres indicated autoimmune pituitary involvement, whereas the presence of APA at low titres was associated with normal pituitary function and morphological findings on magnetic resonance imaging (MRI). Thus, we suggested that APA, when detected at high titres, may be considered a good diagnostic tool to highlight possible occurrence of GHD in adults with autoimmune endocrine diseases. In order to investigate which pituitary hormone-producing cells are the target of these antibodies, the sera of the same patients with idiopathic GHD, either isolated or associated with autoimmune endocrine diseases, were re-tested by a four-layer double indirect immunofluorescence technique.

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Table 1. Characteristics of patients with apparently idiopathic adult GHD isolated (group 1a) or associated with autoimmune endocrine diseases (group 1b) and of autoimmune endocrine patients with normal pituitary function (group 2)

	Group 1a (No. 4)	Group 1b (No. 5)	Group 2 (No. 28)
Sex (F/M)	3/1	4/1	20/8
Age (years)	23 ± 2.5	34.8 ± 3.7	38.1 ± 4.8
APA titre (range)	1/32–1/64	1/32–1/64	1/8
Autoimmune diseases (No.)	0	5	28
HT (No.)	0	4	17
GD (No.)	0	0	9
AD (No.)	0	0	1
CDI (No.)	0	1	1
Hypothalamic-pituitary MRI	Normal (No. 4)	Normal (No. 3) LH/LINH (1) Empty sella (1)	Normal (No. 28)

APA, antipituitary antibodies; HT, Hashimoto's thyroiditis; GD, Graves' disease; AD, Addison's disease; CDI, central diabetes insipidus; MRI, magnetic resonance imaging; LH, lymphocytic hypophysitis; LINH, lymphocytic infundibulo-neurohypophysitis.

Materials and methods

Patients

We studied 37 patients whose sera had been previously tested for APA,²¹ grouped as follows: nine patients, found positive for APA at high titres: 1 : 32–1 : 64 with apparently idiopathic adult GHD; four of them (group 1a: three women, one man; mean age, 23 ± 2.5 years) with isolated GHD diagnosed during childhood without hypothalamic-pituitary disorders. None of them had a past history of cranial trauma or hypothalamic pituitary abnormalities on MRI. All of them had been treated with recombinant GH in childhood until skeletal growth was completed, and then this therapy had been stopped at least 5 years before the reevaluation of GH secretion in adulthood. They had had a good growth response during childhood after GH therapy, without significant differences among them. The remaining five patients (group 1b: four women, one man; mean age, 34.8 ± 3.7 years) had GHD, diagnosed in adulthood, associated with autoimmune endocrine diseases: Hashimoto's thyroiditis (HT, four patients) and central diabetes insipidus (CDI, one patient). Of patients in group 1b, three showed normal imaging, one had MRI characteristics suggestive of lymphocytic adenohypophysitis and possible lymphocytic infundibulo-neurohypophysitis and another had a partial empty sella. Diagnosis of adult GHD was formulated in all patients according to the recommendation of the Growth Hormone Research Society.²² In particular, the response to insulin-induced hypoglycaemia [insulin tolerance test (ITT)] and to arginine was considered impaired with a GH peak less than 6 mU/L and less than 3 mU/L, respectively, as previously described.²¹ According to these criteria, all patients in groups 1a and 1b presented with severe GHD. Moreover, they had serum IGF-I levels below the normal range for age, sex, and nutritional state. Normal basal and dynamic secretion of other pituitary hormones was observed in all nine patients. Finally, a group of 28 patients with autoimmune disease (17 with HT, nine with Graves' disease, one with Addison's disease, and one with CDI), previously found to be APA positive at low titre but with normal pituitary function, were also studied (group 2: 20 women, 8 men; mean age, 38.1 ± 4.8 years). Patients with

hypothyroid HT, Addison's disease, and CDI received appropriate replacement therapy. Hyperthyroid Graves' patients received anti-thyroid drugs.

Characteristics of the groups of APA positive patients are illustrated in Table 1. Antibodies against pituitary hormone-producing cells were evaluated in all sera.

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

Immunological study

Antibodies against pituitary hormone-producing cells were determined by a four-layer double immunofluorescence technique according to Bottazzo *et al.*,^{23,24} with minor modifications using cryostat section of young baboon pituitary gland. In particular, the same cryostat section, in a first immunostaining step, was tested against patient's serum and then fluorescein isothiocyanate (FITC) goat anti-human immunoglobulin sera and in a second immunostaining step against rabbit antisera – anti GH, ACTH, TSH, PRL, LH, FSH, separately, followed by rhodamine goat sera anti-rabbit IgG. The different colour of anti-Ig conjugate against the human sera and against the animal sera, green (FITC) and red (rhodamine), respectively, allowed direct visual assessment of whether the patient's serum and the animal's antihormone serum stain the same or different pituitary cells.

Results

The behaviour of antibodies to pituitary hormone-producing cells in the three groups of APA positive patients is summarized in Table 2 and illustrated in Fig. 1. As regards to group 1a patients, when their sera were tested in conjunction with rabbit antisera to pituitary hormones, APA selectively immunostained GH-secreting cells (Fig. 1). In this group of patients, at the first step, APA immunostained some cells (green cells, A), identified as GH-producing cells when tested with single rabbit antisera against PRL, ACTH, TSH, LH, FSH and GH because all of them were coloured in red only when tested with antisera against GH (red cells, B). Conversely, by the same laboratory

Table 2. Behaviour of antibodies to pituitary hormone-producing cells in patients with apparently idiopathic adult GHD isolated (group 1a) or associated with autoimmune endocrine diseases (group 1b) and in autoimmune endocrine patients with normal pituitary function (group 2)

	Group 1a (No. 4)	Group 1b (No. 5)	Group 2 (No. 28)
GH-producing cell antibodies	+* (4)	++ (5)	+– (28)
PRL-producing cell antibodies	– (4)	– (5)	++ (28)
ACTH-producing cell antibodies	– (4)	– (5)	– (28)
TSH-producing cell antibodies	– (4)	– (5)	– (28)
LH-producing cell antibodies	– (4)	– (5)	– (28)
FSH-producing cells antibodies	– (4)	– (5)	– (28)

+*, Immunostaining selective cells; +, immunostaining some cells; ++, immunostaining many cells; +–, immunostaining very few cells; –, immunostaining no cells.

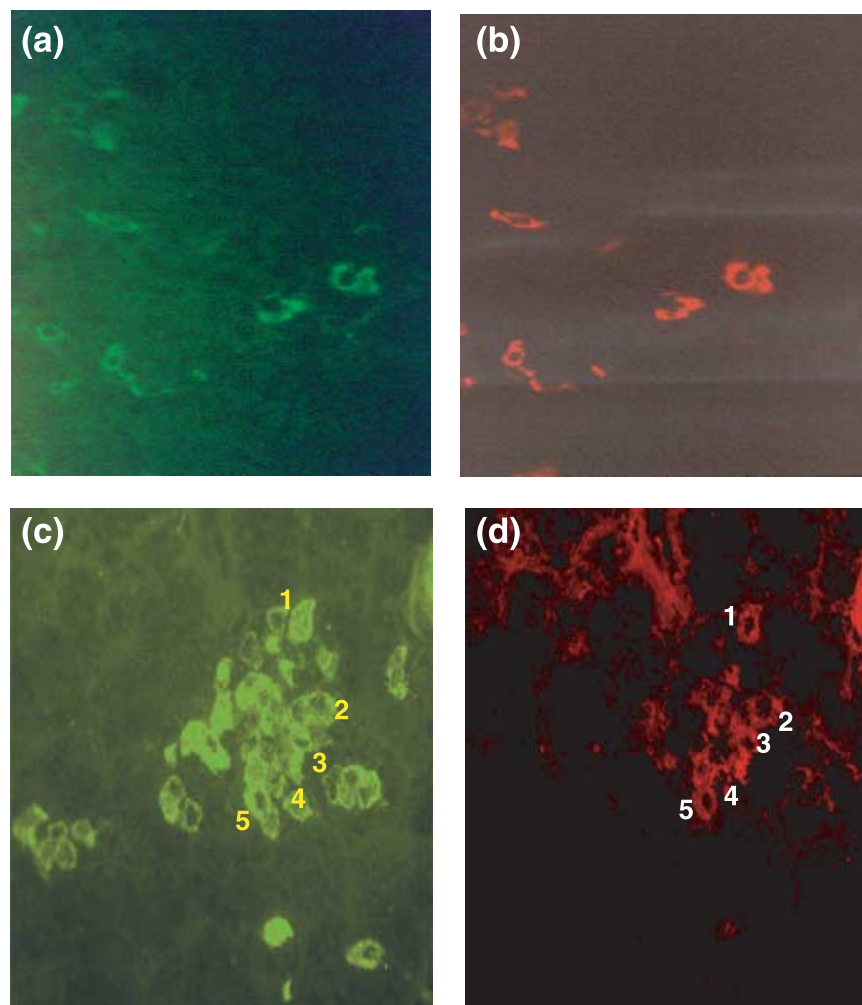


Fig. 1 Immunofluorescence in cryostat sections of young baboon anterior pituitary gland tested against sera of a patient with isolated GHD (a, b). Immunofluorescence in cryostat sections of a patient with GHD associated with other autoimmune diseases (c, d), in a primary step adding FITC goat sera anti-human immunoglobulins (a, c: green colour) and in a second immunostaining step adding rabbit antisera anti-GH followed by rhodamine goat sera anti-rabbit IgG (b, d: red colour). (a) and (b): Correspondence between the cells previously immunostained by APA (a) and those tested with antisera antihuman GH followed by rhodamine goat sera anti-rabbit (b). (c) and (d): The numbers 1–5 indicate which cells among a larger number previously immunostained by APA (c) are immunostained after addition of antigen anti-GH followed by rhodamine goat sera anti-rabbit (d).

protocol, in group 1b patients (Fig. 1), APA immunostained several pituitary cells (green cells, C) resulting only partially as GH-producing cells (red cells, D). As regards group 2 patients, APA immunostained predominantly PRL-producing cells and only rarely some GH-producing cells.

Discussion

Antibodies staining GH-secreting cells were first detected by Bottazzo *et al.*²⁵ by a four-layer immunofluorescence in a girl presenting

with Turner's syndrome, partial GHD and a familial history of autoimmune polyendocrinopathy. These antibodies have also been detected in 3/397 sera of patients with idiopathic short stature²⁶ and in 1/4 patients with idiopathic GHD,¹⁷ suggesting that autoimmunity could play a role in some cases of GHD. So far, further studies into the relationship between antibodies to GH-producing cells and GHD are lacking. For this reason, we re-evaluated by a four-layer double immunofluorescence method the sera of some adult patients with apparently idiopathic GHD, either isolated or associated with autoimmune endocrine diseases, previously found to be positive for APA.

The first important result emerging from this study is that APA selectively recognize GH-producing cells in adult patients with isolated and apparently idiopathic GHD, demonstrating for the first time that somatotrophs are the target of the autoimmune process in these cases.

In a previous study, using an immunoabsorption technique on APA positive sera, we were able to exclude APA being directed against GH itself.²¹ This finding contrasts with previous observations^{27,28} of antihuman GH antibodies in patients with pituitary disorders. However, the different clinical expression of the disease as well as the different time of APA detection with respect to the onset of the disease might explain the conflicting results. In fact, it is well known that serum antibody titres may increase or decrease intermittently, and even disappear in the course of the disease.²⁹ However, as all APA positive patients with apparently idiopathic and isolated GHD had a well-known past history of childhood-onset GHD without hypothalamic-pituitary diseases, we suggest further research into presence of these antibodies, not only in adults, but also in children with idiopathic GHD.

The second remarkable finding emerging from our results is that, in patients with autoimmune endocrine diseases and GHD, APA stained not only GH-producing cells but also other pituitary hormone-secreting cells, even if to a lesser extent. Why these patients clinically express only GHD without other pituitary dysfunction is a matter of concern at the present time. This might depend on the percentage of pituitary cells involved in the autoimmune process: the near total immune involvement of somatotrophs with only partial involvement of the other pituitary hormone-producing cells could account for the selective GH deficiency in these cases.

The different pattern of immunostaining in patients with isolated idiopathic GHD and in those with GHD and autoimmune endocrine diseases is worthy of comment. One could hypothesize the existence of antigens selectively expressed by GH-producing cells in the cases of isolated GHD and antigens shared with other pituitary cells when GHD is associated with other autoimmune endocrine diseases. Whatever the case, the finding of our study allows us to identify two groups of patients with GHD, when the staining of pituitary is restricted to GH-producing cells, they present with an isolated form of GHD, when it is characterised by a diffuse immunostaining of pituitary cells they present with GHD associated with autoimmune endocrine diseases. For this reason, the finding of APA immunostaining of several pituitary cells in GHD patients should prompt a search for other possible autoimmune diseases in subclinical state.

Another point emerging from our results is that APA immunostained predominantly prolactin-producing cells when present at low titres in sera of patients with autoimmune endocrine diseases but normal pituitary function, suggesting that in these cases they could be only a nonspecific marker of pituitary autoimmunity. However, only a careful follow-up of these patients will reveal whether these antibodies represent a risk for the development of pituitary dysfunction.

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