Pituitary autoantibodies
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\textbf{Purpose of review}

The aim of this article is to review recent advancements in pituitary autoantibody assays.

\textbf{Recent findings}

The newest assay is based on the in-vitro transcription and translation of pituitary specific proteins followed by immunoprecipitation with patient sera. The two proteins, PGSF1\textsubscript{a} and PGSF2, were identified as pituitary specific from a human pituitary gland cDNA library. Autoantibodies were found in one patient with biopsy proven lymphocytic hypophysitis and seven with suspected hypophysitis, including idiopathic hypopituitarism. Patients with rheumatoid arthritis, especially if rheumatoid factor negative, also had autoantibodies to PGSF1\textsubscript{a}. An immunoblotting method identified the autoantigen enolase (both \(\alpha\) and neuron-specific), as a marker of neuroendocrine autoimmunity but an in-vitro transcription and translation assay has shown that enolase autoantibodies are nonspecific. Enolase autoantibodies have also been found in Sheehan’s syndrome. Immunoblotting identified a novel 36 kDa pituitary cytosolic autoantigen in adrenocorticotropic (ACTH) deficiency and pituitary membrane proteins of 48, 49 and 43 kDa in patients with lymphocytic hypophysitis. Indirect immunofluorescence using baboon pituitary has been revisited and somatotroph autoantibodies found in patients with idiopathic growth hormone (GH) deficiency. High titre antibodies were thought to be clinically significant. Enzyme-linked immunosorbent assays using human pituitary adenoma cells or rat tissue have identified antibodies in patients with type 1 diabetes, Hashimoto’s thyroiditis and various pituitary disorders but not hypophysitis.

\textbf{Summary}

The search for reliable and specific pituitary autoantibody markers continues.

\textbf{Keywords}

lymphocytic hypophysitis, pituitary autoantibodies, pituitary autoimmunity

\textbf{Introduction}

A sensitive and specific diagnostic assay for pituitary autoantibodies is keenly awaited. Such an assay (or assays) would be particularly useful for clinicians managing patients with atypical pituitary masses, peripartum hypopituitarism or idiopathic hypopituitarism with or without an empty sella [1]. These case scenarios represent the spectrum of autoimmune pituitary disease or lymphocytic hypophysitis, from its acute presentation mimicking a pituitary adenoma, through to sub-acute and chronic forms. The classical sub-acute presentation is a woman in the peripartum period presenting with a pituitary mass and hypopituitarism that spontaneously resolve. Chronic manifestations (e.g. empty sella syndrome) are more difficult to define because such patients rarely, if ever, undergo pituitary biopsy. Until there is a suitable assay, the gold standard for the diagnosis of lymphocytic hypophysitis will remain pituitary biopsy. The recent advancements in the field have centred on the development of new assay techniques incorporating molecular technology and immunoprecipitation. Some new autoantigens have been identified but none as yet are confirmed as completely pituitary specific [2]. Immunoblotting has also identified some novel autoantigens in ACTH deficiency and hypophysitis [3\textsuperscript{**}]. Enzyme linked immunosorbent assay (ELISAs) have been developed in Japan [4,5] and indirect immunofluorescence has been revisited [6] with interesting results in patients with idiopathic growth hormone deficiency. A summary of the techniques and substrates used for their detection is given in Table 1 [2,3\textsuperscript{**},4–7,8\textsuperscript{*},9–23].

\textbf{The concept of ‘organ-specific autoimmune endocrinopathies’}

The autoimmune endocrinopathies are regarded as organ-specific diseases from ‘the traditional’ viewpoint and lymphocytic hypophysitis is considered part of this group. Classic examples are Hashimoto’s thyroiditis, Addison’s disease, type 1 diabetes mellitus and Graves’ disease.

\textbf{Abbreviations}

\begin{tabular}{ll}
ELISA & enzyme linked immunosorbent assay \\
GAD & glutamic acid decarboxylase \\
ICA & islet cell antibody \\
ITT & in-vitro transcription translation \\
\end{tabular}

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1068-3097

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\textbf{Current Opinion in Endocrinology & Diabetes} 2006, 13:344–350

\textbf{Current Opinion in Endocrinology & Diabetes} 2006, 13:344–350

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disease. The corresponding target autoantigens are tissue-specific or cell-specific enzymes: thyroid peroxidase [24], 21-hydroxylase [25], glutamic acid decarboxylase 65 (GAD65) [26], hormones: (insulin) [27] or receptors: thyrotropin receptor (TSHR) [28] respectively. Conversely, ubiquitous antigens may be the target of autoantibodies that are ‘organ-specific’, such as transglutaminase in coeliac disease [29] and pyruvate dehydrogenase in primary biliary cirrhosis [30].

Yet on closer inspection, this concept of ‘specificity’ does not hold completely true. Islet cell antibodies (ICAs) detected by immunofluorescence in patients with type 1 diabetes recognize not only insulin-secreting β cells but react with islet α cells, δ cells, those making pancreatic polypeptide [31] and multiple pituitary cells [32]. Pre-absorption of these sera with GAD and islet cell antigen (IA2) does not abolish ICAs suggesting that there are other relevant islet cell autoantigens that are of sialoglycolipid nature [33]. In Hashimoto’s thyroiditis, patients can rarely develop an encephalopathy picture that appears to correlate with their thyroid autoantibody status and not some concurrent neurological condition [34,35]. Patients with Graves’ disease have been shown to have antibodies that cross-react with pituitary cells [36].

Translating ‘traditional’ logic to pituitary autoimmunity or lymphocytic hypophysitis, the target autoantigens should be cell-specific enzymes, hormones or receptors. The enzymes in the pituitary are present in hypothalamic tissue and neuroendocrine tissues throughout the body, for example neuron-specific enolase [14], prohormone convertase 1/3, neuroendocrine protein 7B2, [23] and the family of carboxypeptidases [37]. Although we identified enolase as a target autoantigen in lymphocytic hypophysitis, reactivity is found in the sera of many other patients, including 20–46% of those with pituitary adenoma [9,21] and at low titres in 5–10% of normal controls. Proteins found to be pituitary-specific (PGSF1a and PGSF2) from a human pituitary cDNA library [2] were targeted by sera from some Japanese patients with hypophysitis and hypopituitarism [38]. Subsequent experiments showed that PGSF1a was also recognized by sera from patients with rheumatoid arthritis [22]. There is past and recent evidence that growth hormone can be a target autoantigen [12,17] but there is no body of work on any receptors as potential targets in hypophysitis.

Finally, different arms of the immune system interact with target antigens in different ways. Epitopes are the components of antigen that are recognized by the immune system. Epitopes are recognized as linear epitopes by T cells, whilst conformational (‘three-dimensional’) structures are the typical target of B cell or autoantibody recognition. Critically, the major ‘endocrine’ autoantigens, thyroid peroxidase [24], adrenal 21-hydroxylase [25] and GAD65 [26], all have such complex three-dimensional binding sites. In addition, the sites recognized by patient

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Table 1  Studies on pituitary autoantibodies published in the last 10 years

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<thead>
<tr>
<th>Technique</th>
<th>Tissue substrate</th>
<th>References</th>
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<tr>
<td>Indirect immunofluorescence</td>
<td>Rat pituitary</td>
<td>Fetissov et al. 2002 [7]</td>
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<td>Immunoblotting</td>
<td>Human autopsy cytosolic or membrane tissue preparations</td>
<td>Strömberg et al. 1998 [10]</td>
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<td>Takao et al. 2001 [12]</td>
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<td>O’Dwyer et al. 2002 [14]</td>
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<td>Bensing et al. 2004 [16]</td>
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<td></td>
<td>Rat tissue preparation</td>
<td>Bensing et al. 2005 [3*]</td>
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<td></td>
<td>Porcine tissue preparation</td>
<td>Yabe et al. 1998 [5]</td>
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<td>ELISA</td>
<td>Human adenoma cell line</td>
<td>Kobayashi et al. 1997 [19]</td>
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<td>Rat pituitary cells</td>
<td>Kobayashi et al. 1998 [20]</td>
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<td>Kikuchi et al. 2000 [17]</td>
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<td>Nishino et al. 2001 [18]</td>
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<td>Keda et al. 2002 [4]</td>
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<td>Tatsumi et al. 2003 [23]</td>
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ELISA, enzyme linked immunoabsorbent assay; ITT, in-vitro transcription translation.
sera are different from those recognized by normal control sera at low titres [39]. The significance of this differential epitope mapping is unclear.

The latest assays in development will be discussed in the context of the above comments, including a brief section on technical issues in the laboratory.

**In-vitro transcription translation and immunoprecipitation of pituitary proteins**

The newest assay for pituitary autoantibodies involves the production of recombinant pituitary proteins *in vitro* using rabbit reticulocyte lysate. Methionine residues in the proteins are labelled with $^{35}$S and the proteins then used in an immunoprecipitation step with patient sera and protein-A sepharose [40]. This approach implies that the target autoantigen has already been identified in some way. Tanaka et al. [2] chose two novel pituitary gland specific factors, PGSF1a and PGSF2, as potential candidates because of their pituitary specific tissue expression. They also tested enolase [21] and growth hormone in this system on the basis of our original immunoblotting results [9] and on previous work from Japan, identifying growth hormone as the 22 kDa protein autoantigen on immunoblots [12]. Subsequently, other potential candidates, the pro hormone-processing enzymes prohormone convertases 1/3 and 2, carboxypeptidase E (CPE) and prohormone convertase 2 regulatory protein 7B2 were studied [23]. Again, these are not pituitary specific.

One of three patients with biopsy proven hypophysitis and two patients with isolated ACTH deficiency had positive antibody indices to PGSF1a. Further studies have shown that PGSF1a can also be a target autoantigen in rheumatoid arthritis [22], particularly in patients with more erosive disease and in four of eight patients who were rheumatoid factor negative. This assay appears promising but studies of pituitary function in rheumatoid patients are needed to confirm the specificity of the assay. Two of 14 patients with suspected hypophysitis or infundibulohypophysitis and three of 14 patients with hypopituitarism had reactivity to PGSF2. Anti-growth hormone antibodies were detected in four patients with suspected hypophysitis or hypopituitarism and in two patients with other autoimmune diseases, but the antibody indices were relatively low with none above two. No patients with a pituitary adenoma had positive indices to any of these autoantigens [2]. Two of 14 patients with lymphocytic hypophysitis had a PC1/3 autoantibody index over 1.5 but so did five of 11 patients with nonfunctioning pituitary macroadenoma [23].

A separate publication by Tanaka et al. [21] looked at enolase in the in-vitro transcription translation (ITT) assay. They confirmed similar results to our immunoblotting study, demonstrating positive autoantibodies in 41% of patients with lymphocytic hypophysitis, 20% with other autoimmune diseases and 4.3% of healthy controls but found even higher levels (46% versus 20% with immunoblotting) of autoantibodies in patients with pituitary adenoma. Reactivity to enolase cannot be used as a specific marker in pituitary autoimmunity but such a high level in tumour patients also raises the question of its use as an indicator of an autoimmune diathesis.

Theoretically, the ITT assay could be used to express any number of possible target autoantigens. Some autoantigens, such as GAD in type 1 diabetes, can best be detected by immunoprecipitation [26], as they are not recognized by patient sera in their denatured form. ITT has, however, been problematic with certain proteins. In the case of the thyrotropin receptor (TSH), it was possible to obtain adequate amounts of protein but not the normal highly glycosylated form which is required for conformational binding of Graves’ sera [41]. The addition of canine pancreatic microsomes had been used by Li et al. [42] to improve production, and presumably folding, of the autoantibody reactive calcium-sensing receptor but was ineffective for TSHR. Expression of adequate amounts of bioactive TSH receptor could only be obtained after transfection into a leukaemia cell line [43]. Future studies with potential pituitary target autoantigens will need to consider these technical issues.

**Immunoblotting**

The immunoblotting assay was developed to provide an alternative approach to immunofluorescence [44]. Immunoblotting identifies target autoantigens based on linear epitopes and characterizes them by molecular weight. The proteins are in a denatured form and there may be hundreds of proteins represented at any particular molecular mass on a gel. Nevertheless, this technique enables the purification of relevant target proteins using column chromatography and immunoblotting of each protein fraction as was described with enolase [14]. It can also be used to characterize isoforms on two-dimensional gels [15]. In this latter paper, serum from a peripartum woman with lymphocytic hypophysitis recognized neuron-specific enolase in both the placenta and pituitary. This observation provides an intriguing link to the frequent presentation of lymphocytic hypophysitis in pregnancy and to a recent study in Sheehan’s syndrome [13]. In a large series of Indian women with true Sheehan’s syndrome Goswami et al. [13] found 12 of 19 or 63.1% had developed antienolase antibodies compared with 17.8% (five of 28) of women with normal pregnancies and 14.2% (four out of 28) of women who had never conceived. The evolution of their hypopituitarism was often over many years rather than immediately postpartum, which supported the theory that the insult
at the time of pregnancy triggered a subsequent autoimmune process.

In 2001, Takao et al. [12] identified autoantibodies to a 22 kDa human pituitary cytosolic protein in 73% or 11 of 15 patients with lymphocytic hypophysitis and 77.8% or seven of nine patients with isolated ACTH deficiency. Sequencing of this protein showed it to be growth hormone. Interestingly nine of the 11 patients with positive results had growth hormone deficiency on formal testing, supporting a pathogenic role for these antibodies. Kobayashi et al. [20] made the interesting observation that preabsorption with pancreatic antigens, but not liver, spleen or kidney extracts, abolished pituitary autoantibody reactivity to the 22 kDa protein. This data correlates well with Bottazzo’s observations [32] in patients with diabetes and positive ICA, whose sera also cross-reacted with pituitary proteins.

Recent studies have identified a novel 36 kDa pituitary cytosolic autoantigen in patients with ACTH deficiency (12 of 65 or 18.5% versus two of 57 or 3.5% in healthy controls, statistically significant \( P < 0.021 \)) [3**]. ACTH deficiency is a prominent feature of lymphocytic hypophysitis and isolated ACTH deficiency is known to co-exist with several other autoimmune disorders [45]. In this large series of patients collected over many years in Poland, 61 of 65 had isolated ACTH deficiency, 51% had another autoimmune disease and 85% (55 of 65) had positive thyroid autoantibodies [3**]. Patients with autoantibodies to the 36 kDa protein had a higher frequency of thyroglobulin autoantibodies than the patients who were not immunoreactive to the 36 kDa protein. Studies in other pituitary diseases and identification of the 36 kDa autoantigen are necessary before further conclusions can be drawn from these results.

The empty sella syndrome is not a homogenous entity and in some cases it may represent the fibrotic end-stage of lymphocytic hypophysitis. Bensing et al. [16] described a group of patients with empty sella syndrome who did not have evidence of high titre pituitary autoantibodies. The fascinating observation in this group was that 15 patients had Type 2 diabetes or impaired glucose tolerance and a body phenotype of central obesity. One could speculate that these patients represent a phenotype of a hypothalamic syndrome with centrally mediated diabetes and secondary pituitary atrophy, but not underlying autoimmunity.

Studies looking at human pituitary membrane antigens are limited. Nishiki et al. [11] identified specific antibodies to 68, 49 or 43 kDa proteins in five of 13 patients with lymphocytic hypophysitis, one of 12 patients with infundibuloneurohypophysitis and none of four patients with isolated ACTH deficiency. These proteins are of interest but have not yet been further characterized.

There are several technical considerations with immunoblotting. The quality of the pituitary tissue preparation is critical. Autopsy tissue taken more than 24 h post mortem is likely to be markedly autolysed and to give poor results. Dissection and homogenization of tissues needs to be done at 4°C and in the presence of a cocktail of anti-proteolytic enzymes [44]. In the centrifugation process, the initial low speed pellet containing nuclear debris and mitochondria is discarded, so any autoantigen in this fraction would be lost. The pituitary contains at least five different hormone secreting cell types and some such as thyrotrophs and corticotrophs, make up less than 5–10% of the gland volume. Conceivably, a relevant target autoantigen from one of these cell types may be present in such small quantities in pituitary tissue preparations as to be undetectable.

Finally, the quality of both primary and secondary antibodies is important. Primary antibodies are those in patient sera and they tend to give very high background activity if the sera have not been stored optimally (personal observation).

**Indirect immunofluorescence**

In 1975 Bottazzo et al. [46] first described autoantibodies to pituitary prolactin-secreting cells using indirect immunofluorescence in 19 of 287 patients with endocrine autoimmunity but no hypopituitarism. In general, the titre of pituitary autoantibodies found by immunofluorescence was low. The immunofluorescence assay has been the most widely used technique but very few patients with biopsy-proven and suspected lymphocytic hypophysitis have been studied and the results have been particularly disappointing and unilluminating. Immunofluorescence recognizes the conformational structure of antigens and has the advantage of identifying the pituitary cell type and subcellular structures that are targeted by pituitary autoantibodies, but it cannot identify the target autoantigen proteins themselves.

The choice of pituitary substrate is critically important but problematic. Although fresh human tissue would be ideal, the ethical issues of using fetal glands and the limited supply of surgical tissue make this untenable. Bottazzo’s original publications concluded that baboon pituitary was the most suitable alternative [46]. A detailed species specificity study outlining the problems of heterophile antibodies was published by Gluck and Scherbaum in 1990 [47]. Human sera positive for pituitary autoantibodies on human fetal substrate were only recovered 4% with adult baboon, 0% with fetal cymolgous monkey, 20% with porcine, 11% with bovine,
11% with ovine and 7% with rat tissue, suggesting the use of animal tissue produced results with no clinical significance. Heterophilic antibodies to the animal substrates were also detected at a rate of 4–15%. The extent of this nonspecific species cross-reactivity can also be seen in immunoblotting experiments [9]. Important autoantigens, however, usually are conserved across species.

Recently De Bellis and colleagues [6] have revisited indirect immunofluorescence using cryostat sections from young baboon pituitary glands. They found high titre pituitary autoantibodies in 33% of patients with idiopathic GH deficiency and low titres in six of 20 patients with adenoma. Twenty-two percent of patients with autoimmune endocrine diseases had antibodies (40/180) of whom five had high titres. High titres were universally associated with severe isolated GH deficiency and the target cells were the somatotrophs, whereas low titres appeared to have no effect on pituitary function.

**Enzyme linked immunoabsorbent assay**

An ELISA was first developed in Japan for pituitary autoantibody detection [5]. Using rat pituitary homogenate as an antigen source, pituitary autoantibodies were detected in patients with type 1 diabetes [5], autoimmune thyroiditis [18] as well as various pituitary disorders [17]. This research group has also found the prevalence of pituitary autoantibodies to be significantly higher in type 2 diabetes patients than in control subjects using porcine instead of rat pituitary as antigen [20].

Keda et al. [4] measured autoantibodies to cell surface antigens of human pituitary adenoma cells and rat pituitary cells with a cellular variant of an ELISA. In this study, patients with idiopathic hyperprolactinemia or idiopathic isolated GH deficiency had autoantibodies more frequently to prolactin-secreting cells and GH-secreting cells respectively, than patients with other forms of pituitary diseases.

No sera from biopsy-proven lymphocytic hypophysitis patients have been tested using ELISA methodology.

**Autoantibodies to pituitary hormones**

In the original immunoblotting method paper [44], preabsorption studies showed that in children with GH deficiency, pituitary membrane and cytosolic autoantibodies were not targeting growth hormone itself. De Bellis et al. [6] showed that 33% of patients with isolated GH deficiency of childhood onset, had somatotroph cell, but not GH, autoantibodies.

Nevertheless, there are a number of studies showing that pituitary hormones themselves can be targets, just as insulin is a recognized autoantigen in type 1 diabetes. Mau et al. 1993 [48], demonstrated anti-ACTH and anti-growth hormone antibodies in two of six patients with empty sella syndrome and anti-ACTH and anti-TSH antibodies in three of five patients with pituitary tumours. No antibodies were found in six controls and there was no correlation with hormonal status. Autoantibodies reacting with ACTH have also been reported in patients with eating disorders as well as in some healthy controls [7].

Immunoblotting studies from Kikuchi et al. [17] and Takao et al. [12] identified a 22 kDa protein as a target autoantigen and they subsequently showed this to be growth hormone. To our knowledge there are no similar studies looking at prolactin.

The autoantigens that have been characterized so far are summarized in Table 2.

**Conclusion**

That a single diagnostic assay will cover the broad clinical spectrum of lymphocytic hypophysitis is unlikely. There is strong evidence that there are multiple autoantigens in lymphocytic hypophysitis. The pituitary autoantibodies in a patient with lymphocytic hypophysitis and isolated ACTH deficiency are almost certainly going to be different to those from a patient with isolated TSH deficiency or panhypopituitarism and the empty sella syndrome. The challenge is to match the target autoantigens with each scenario.

**Acknowledgements**

We thank Professor Rodney Scott and Dr Glenn Reeves for constructive comments on the manuscript.
References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 401).


The authors describe the identification of a novel 36kDa pituitary cytosolic autoantigen in a large series of patients with isolated ACTH deficiency and other evidence of autoimmunity.


This editorial discusses the work by Pietropaolo et al. [33]. It provides an interesting analysis of the current state of play of ICA assays and diabetes prediction.


This describes an important study from the Pittsburgh Paediatric Endocrinology group, which shows that limiting islet autoantibody assays to GAD, IA2 and insulin, will miss other islet reactivities that may be more predictive of diabetes risk. It highlights the fact that islet cell autoantibodies recognize multiple autoantigens that are not necessarily β-cell specific.


