Autoimmunity and the pituitary

THEKLA MAUERHOFF
RITA MIRAKIAN
GIAN FRANCO BOTTAZZO

Since thyroid autoimmunity was described more than 30 years ago (Roitt et al, 1956), several other endocrine organs have been found to be vulnerable to the same pathogenetic mechanism. However, it was only in 1962 that Goudie and Pinkerton described the first case of lymphocytic hypophysitis in a woman with Hashimoto’s thyroiditis and proposed that autoimmunity could also play a role in affecting the pituitary gland. If the existence of lymphocytic hypophysitis was a recent finding, almost ten years elapsed before autoantibodies to pituitary endocrine cells were reported (Bottazzo et al, 1975). Compared to other endocrine organs, autoimmune disease of the pituitary appears to be rare, but its existence as a separate pathological entity is now generally accepted, on both clinical and experimental grounds. Major advances in the field have been hampered by the lack of histological confirmation in patients with ‘idiopathic’ pituitary deficiencies and by the scant availability of normal human pituitary tissue for laboratory tests and more sophisticated investigations.

LYMPHOCYTIC HYPOPHYSIS

Since the first case report (Goudie and Pinkerton, 1962), an increasing number of patients have been found to have an extensive lymphocytic infiltration of the anterior pituitary surrounding atrophic acini, although inevitably many more patients have remained undiagnosed. In six cases, the lymphoid tissue was organized in follicles with defined germinal centres (Lack, 1975; Quencer, 1980; Portocarrero et al, 1981; Cebelin et al, 1981) and the picture resembled the classical histological pattern of Hashimoto’s thyroiditis or that described in other endocrine glands affected by autoimmune. In three more recent cases, the pituitary was studied by electron microscopy (Asa et al, 1981; Baskin et al, 1982; McKell, 1983), and it was shown that in heavily infiltrated areas pituitary cells were in intimate contact with lymphocytes and plasma cells, thus confirming that an immune attack was actually occurring.

Infiltration at the level of adenohypophysis is in sharp contrast to the absence of inflammation in the pars posterior of the gland. It has been
known for a long time that a moderate lymphocytic infiltrate could be
detected in a proportion of normal pituitaries studied at autopsy from
patients who died without overt pituitary disease (Simonds and Brandes,
1925; Shanklin, 1951). However, these findings were restricted always to the
region equivalent to the pars intermedia in rodents or to the pars posterior
and they must not be confused with classical adenohypophysitis.

Most of the reported cases were in young women during pregnancy or in
the postpartum period and they were affected by various symptoms of
hypopituitarism (see Table 1). Six patients died of pituitary insufficiency and
adenohypophysitis was detected at autopsy.

However, since 1980 this pituitary lesion has been increasingly diagnosed
after hypophysectomy, predominantly in patients who presented with a
wide range of symptoms related to pituitary hyofunction. Nine subjects,
mostly women during pregnancy or after delivery, had signs of compression
of the visual pathways or headache. A pituitary tumour was the tentative
diagnosis, but surgical exploration revealed instead the presence of an
extensive lymphocytic infiltration in the gland.

It is interesting to recall that in five cases the pattern of florid hypophysitis
was associated with hyperprolactinaemia amenorrhoea and/or galactor-
roe (Quencer, 1980; Portocarrero et al, 1981; Cebelein et al, 1981; Asa et
al, 1981; Mazzone et al, 1983). These symptoms persisted for several months
after delivery and the diagnosis of prolactinoma was proposed before hypo-
physectomy. The initial clinical presentation was subsequently interpreted
as the result of the compression of the hypothalamohypophysial stalk
through an inflamed and enlarged pituitary. Alternatively, it is attractive to
postulate the existence of an autoimmune interaction at the site of the
surface receptors on the prolactin (PRL)-producing cells. By analogy with
thyroid stimulating hormone (TSH) receptor antibodies found in Graves' disease,
similar specificities could act in some cases of hyperprolactinaemia,
thus being responsible for the final clinical outcome.

The fact that all the patients were women and the symptoms first appeared
during pregnancy or in the postpartum period stimulates other interesting
speculations. During pregnancy there is an increase in size of the pituitary
gland with physiological hyperplasia of the PRL-producing cells (Erdheim
and Stumme, 1909). This could induce an increased breakdown of cellular
products into the circulation, with consequent neo-antigen liberation which,
in turn, could trigger an autoimmune reaction in predisposed individuals.

On the other hand, as convincingly shown in autoimmune thyroiditis
(reviewed by Belfiore and Bottazzo, 1987), pregnancy and the postpartum
period themselves appear to affect the immune system of the mother and
this could in some way help to initiate or aggravate the autoimmune attack at
the level of the pituitary gland. The importance of pregnancy and its
relationship to autoimmunity is stressed by the finding of Engelberth and
Jezkova (1965) who showed that 18% of sera from a population of
apparently normal women reacted against an adenohypophysis extract
seven days after delivery. Interestingly, in an experimental model the
degree of hypophysitis in immunized animals was increased in severity in the
postpartum period (Levine, 1967).
### Table 1. Cases of lymphocytic hypophysitis.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Sex/age (years)</th>
<th>Clinical history</th>
<th>Associated disorders</th>
<th>Autopsy or histology finding</th>
<th>Other investigations</th>
</tr>
</thead>
</table>
| Goudie and Pinkerton (1962) | ♀ 22            | Onset of symptoms four months post-partum, goitre, hypotension, amenorrhoea last six months of life, death from acute gangrenous appendicitis | Hashimoto's thyroiditis                      | Autopsy:  
  —atrophy of the pituitary  
  —lymphocytic infiltration | —                    |
| Hume and Roberts (1967) | ♀ 74            | Menopause at 50, hypotension, pallor, unconsciousness                             | Pernicious anaemia, chronic atrophic gastritis, focal lymphocytic thyroiditis | Autopsy:  
  —atrophy of the pituitary  
  —lymphocytic infiltration | —                    |
| Egloff et al (1969)      | ♀ 29            | Onset of symptoms four weeks postpartum, amenorrhoea, hypoglycaemia               | —                                            | Autopsy:  
  —enlarged pituitary            | —                    |
| Lack (1975)         | ♀ 42            | Two miscarriages, schizophrenia, weight loss, anaemia, dehydration, loss of body hair | Mild lymphocytic infiltration of the parathyroid and adrenals, pulmonary granulomas, hypersensitivity type of necrotizing vasculitis | Autopsy:  
  —normal size pituitary         | —                    |
| Gleason et al (1978)  | ♀ 59            | Five years of post-hysterectomy hypoglycaemia during last year of life            | Severe arthralgias                           | Autopsy:  
  —enlarged pituitary            | —                    |
<table>
<thead>
<tr>
<th>Authors</th>
<th>Sex/age (years)</th>
<th>Clinical history</th>
<th>Associated disorders</th>
<th>Autopsy or histology finding</th>
<th>Other investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayfield et al (1980)</td>
<td>♀ 23</td>
<td>Onset of symptoms seven months post partum, visual disturbances, weight loss, hypoglycaemia</td>
<td>—</td>
<td>Hypophysectomy: —enlarged pituitary —inflammatory cells —presence of IgG+ cells —no deposit of immunoglobulins</td>
<td>HLA Bw35 autoantibodies against several pituitary cells</td>
</tr>
<tr>
<td>Richtsmeier et al (1980)</td>
<td>♀ 31</td>
<td>Onset of symptoms two weeks post partum, death three months post partum, hypercalcaemia, anorexia</td>
<td>Chronic lymphocytic thyroiditis, parathyroid hyperplasia</td>
<td>Autopsy: —atrophy of the pituitary</td>
<td>—</td>
</tr>
<tr>
<td>Quencer (1980)</td>
<td>♀ 25</td>
<td>Headache five months post partum, galactorrhoea, amenorrhoea</td>
<td>Hyperprolactinaemia</td>
<td>Hypophysectomy: —enlarged pituitary</td>
<td>—</td>
</tr>
<tr>
<td>Asa et al (1981)</td>
<td>1) ♀ 28</td>
<td>Onset of symptoms at six months of gestation, visual disturbances</td>
<td>Gestational diabetes</td>
<td>Hypophysectomy: —enlarged pituitary</td>
<td>HLA Bw35 —invading lymphocytes Pituitary autoantibodies</td>
</tr>
<tr>
<td></td>
<td>2) ♀ 29</td>
<td>Onset of symptoms at seven months of gestation, amenorrhoea and inability to lactate in the post partum, headaches, vomiting, weight loss, lethargy, loss of body hair and libido</td>
<td>Hyperprolactinaemia</td>
<td>Hypophysectomy: —enlarged pituitary</td>
<td>Anti-mitochondrial antibody, no immune complex deposits</td>
</tr>
<tr>
<td>Authors</td>
<td>Age</td>
<td>Symptoms</td>
<td>Diagnosis</td>
<td>Procedure</td>
<td>Findings</td>
</tr>
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</tr>
<tr>
<td>Cebelin et al (1981)</td>
<td>22</td>
<td>Galactorrhoea four months post partum, suicide</td>
<td>Hyperprolactinaemia</td>
<td>Autopsy: enlarged pituitary</td>
<td>—</td>
</tr>
<tr>
<td>Baskin et al (1982)</td>
<td>33</td>
<td>Onset of symptoms during the eighth month of pregnancy, decreased visual acuity</td>
<td>Hyperprolactinaemia</td>
<td>Hypophysectomy: enlarged pituitary</td>
<td>Electron microscopy — activated lymphocytes interdigitating with degenerating secretory cells</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>Onset of symptoms three weeks post partum, headache and visual disturbances</td>
<td>—</td>
<td>Hypophysectomy: enlarged pituitary</td>
<td>—</td>
</tr>
<tr>
<td>Mazzone et al (1983)</td>
<td>37</td>
<td>Onset of symptoms during the sixth month of pregnancy, headache, anorexia, exhaustion, amenorrhoea, hypotension</td>
<td>Hyperprolactinaemia, pernicious anaemia</td>
<td>Hypophysectomy: enlarged pituitary</td>
<td>Anti-parietal cell antibodies</td>
</tr>
<tr>
<td>McKell (1983)</td>
<td>30</td>
<td>Three years of progressive hypopituitary and died after cholecystectomy</td>
<td>Chronic lymphocytic thyroiditis</td>
<td>Autopsy</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>Five years of amenorrhoea and progressive adrenal and thyroid failure</td>
<td>—</td>
<td>Hypophysectomy: — sclerotic fibrotic pit — scattered granulomas with giant cells, foci of neutrophilic and lymphoplasmacytic infiltration</td>
<td>Electron microscopy — activated lymphocytes interdigitating with degenerating secretory cells</td>
</tr>
</tbody>
</table>
As for the other, more common, autoimmune endocrinopathies, most cases of lymphocytic hypophysitis were found associated with multiple autoimmune disorders and/or with the presence of circulating organ-specific autoantibodies (Table 1). Among these disorders, the most commonly represented was Hashimoto’s thyroiditis, but other related conditions were often associated (Sobrinho-Simoes et al., 1985). Surprisingly, insulin-dependent diabetes is not a common finding in these patients, although one case was described with gestational diabetes (Asa et al., 1981, case 1, shown in Table 1).

In most patients the correct diagnosis was proven at autopsy or surgery, but there are reports in which it was tentatively reached on clinical grounds and from appropriate laboratory investigations. Notterman et al. (1984) reported a 32-week pregnant woman with hypoglycaemic seizure and coma of hypopituitary origin, but this condition was not associated with other autoimmune diseases. On the other hand, Gossain and Rouner (1984) have described a 23-year-old woman with inability to lactate and severe weakness in the postpartum period. This patient had PRL and adrenocorticotropic hormone (ACTH) deficiency with autoimmune thyroiditis; Addison’s disease was recorded in one of her first-degree relatives.

In the past few years, clinicians have become more aware of the existence of lymphocytic hypophysitis, aided by an increasing appearance in the literature of case reports describing patients with typical clinical manifestations and histology finally proven. Thus, a correct diagnosis should be suspected when pituitary insufficiency appears in young women during or just after pregnancy, with or without galactorrhoea. The computed tomography (CT) findings are not usually of great help since the sella is enlarged most of the time and when a contrast-enhancing mass is detected (six out of seven cases investigated with CT scan showed this pattern) this can lead to a misdiagnosis of pituitary adenoma. The presence of clinical autoimmune manifestations or the detection of organ-specific autoantibodies in the patients and/or in their relatives supports the possibility of an autoimmune mechanism underlying the pituitary symptoms. Therefore an enlarged autoantibody screening, including the search for specific antibodies against pituitary cells, should be carefully sought in these patients.

POLYGLANDULAR AUTOIMMUNE SYNDROMES: THE PITUITARY INVOLVEMENT

The clustering of endocrine autoimmune disorders, either in the same individual or in their family members, has been known for a long time (reviewed by Bottazzo and Doniach, 1985), but, as mentioned, the inclusion of the pituitary in this constellation is only a recent acquisition. This is due to several factors: hypophysitis is a somewhat unusual finding, there is probably a long asymptomatic period of latency before the onset of clinical manifestations and some difficulties still exist in obtaining specimens for confirmatory histological examination.

Saleh (1984) described a 48-year-old man with pernicious anaemia,
primary hypothyroidism and isolated ACTH deficiency. This patient had, as expected, antibodies to gastric parietal cells and thyroid microsomes but, in addition, his serum stained ACTH-producing cells when tested on pituitary sections by an indirect immunofluorescence (IFL) technique. However, as it will be described in detail below, specific autoantibodies to ACTH-cells are not easily identifiable unless fragments of immunoglobulins are selectively cleared. In two more recent cases of isolated gonadotrophin hormone failure associated with type II polyglandular autoimmune syndrome (Barkan et al, 1985; reviewed in Chapter 6), the authors conclusively showed that the gonadotropin deficiency was due to a selective pituitary failure. However, antibodies against endocrine pituitary cells were not found, although anti-adrenal and islet cell antibodies (ICA) were present in one patient and anti-adrenal and anti-thyroid antibodies in the other. Although no histological confirmation was obtained, there was the suggestion that the pituitary abnormality was due to an autoimmune attack.

By performing regular functional and stimulatory tests in polyendocrine autoimmune patients, one can predict that more cases will be identified showing selective pituitary hormone defects, indicating that the pituitary gland is more often involved in the spectrum of the polyglandular autoimmune syndrome than previously suspected (for a review of pituitary hormone defects, see Vlasveld et al, 1984).

DETECTION OF PITUITARY CELL ANTIBODIES

Technical aspects

The first attempt to identify antibodies against pituitary cells was more than 20 years ago when Engelberth and Ježkova (1965) published preliminary positive results in a prospective study using a complement consumption test in which a crude extract of adenohypophysis was used as a substrate. Surprisingly, 10 years elapsed before the first demonstration of pituitary antibodies by an indirect IFL technique (Bottazzi et al, 1975). Since then, this test has become the assay of choice for the detection of these specificities in our own and several other laboratories.

Undiluted serum is first applied to cryostat sections (4 μm thick) of fresh frozen pituitary glands, followed by the conventional sandwich technique employing fluorescein/isothiocyanate conjugates to human IgG, IgA, IgM or C3. The latter reagent is applied after a complement source, fresh normal human serum (NHS), is incubated on the section following the test serum (Bottazzi et al, 1980a). When a positive reaction is detected, a double fluorochrome technique is employed on the same section to identify the type of pituitary cell which reacts with the patient’s autoantibody. In this case, the appropriate anti-hormone antiserum is incubated as a third layer followed by a rhodaminated conjugate, carefully chosen to avoid cross-reaction with the first two layers. To achieve this, essential controls include sections stained with one or the other serum and both conjugates to detect nonspecific interspecies reactions. For final confirmation of the selective positive staining on certain cells, double-exposure photographs are taken in
which cells stained by both conjugates will appear orange, while the others remain separate in green and red (Bottazzo et al, 1980c).

Choice of tissue

In our experience, the best substrate was found to be human pituitary gland obtained at hypophysectomy for advanced carcinoma of the breast, but this source of tissue is no longer available. At present, fresh postoperative pituitaries constitute the main bulk of tissue for routine and research screening programmes, but sometimes the small fragments obtained through nasal suction may lack some relevant endocrine cell populations. Pretesting the blocks with the corresponding hormone anti-sera is essential before embarking on the final test. Human postmortem pituitaries are not very reliable material, probably because of the time lapse before autopsy and the stress agony. Fetal glands (12th to 22nd week of gestation) recently have been found suitable for research in pituitary autoimmunity (Scherbaum et al, 1987), although the small size of the specimens constitutes a not irrelevant limiting factor.

Pituitaries from rodents have given controversial results with a poor degree of concordance among laboratories. These substrates often detect heterophile antibodies and NHS is often positive (R. Mirakian, personal communication). Despite all these technical problems, it remains to be clarified why glands obtained from prethyroidectomized castrated guinea pigs detect certain types of pituitary antibodies in human sera which otherwise do not stain pituitaries from untreated animals (Pouplard et al, 1980b). Pituitary from primates is the second best choice and results using human and baboon pituitaries are concordant in the majority of positive sera tested (Pouplard et al, 1985).

Characteristics of cells recognized by pituitary antibodies

Pituitary antibodies are directed against cellular components but not against the synthesized hormone itself. This has been firmly established by absorption and inhibition of binding studies (Bottazzo et al, 1975). Sometimes sera of patients treated with exogenous injections of pituitary hormones (e.g. growth hormone, GH) may stain the corresponding cells, but again absorption studies enable clarification of the true nature of the autoantibody reaction. So far, most of the antibodies detected in the patient sera are directed to PRL-producing cells, although isolated cases with antibodies against GH (Bottazzo et al, 1980b) or gonadotrophin-producing cells were also described (Pouplard, 1984).

The study of autoantibodies selectively directed against ACTH cells presents an important technical problem. This is because normal human immunoglobulins spontaneously bind to these cells through their Fc portion (Pouplard et al, 1976). It is therefore not possible to prove the existence of autoantibodies to ACTH-cells unless sera are subjected to pepsin digestion and the Fab fragment preparations only used for staining the sections. ACTH cells in fetal pituitaries apparently do not have Fc receptors and this tissue can be safely employed for the screening of patients with idiopathic
ACTH deficiency and for the detection of ACTH-cell antibodies as possible predictive markers for relapse after pituitary microsurgery for Cushing's disease (Scherbaum et al., 1987).

Patterns of IFL staining

Two distinct patterns have been described using human pituitary as a substrate: the first is characterized by a granular cytoplasmic staining confined to a single cell type, mostly PRL-producing cells (Figure 1) and, more rarely, GH-producing or gonadotropin cells. In the second, the stain diffuses through the acini to involve the majority of the cells in the gland, the so called 'multiple pattern' (Figure 2) (Mirkian et al., 1982).

Pituitary antibody isotypes and titres

The specificities are usually IgG or IgA class but occasional IgM reactivity can also be detected (see below). The titre of the antibodies is generally low and in half of the cases the positive IgG is complement fixing.

PITUITARY ANTIBODIES AND CLINICAL CORRELATIONS

If the existence of pituitary antibodies is well recognized, it is still difficult to establish their precise clinical relevance. In addition, the lack of standardization of the detection techniques does not facilitate interlaboratory comparisons.

In the study by Engelberth and Jezkova (1965) 128 women were screened for autoantibodies to adenohypophysis. From the time of delivery until the fifth day postpartum, none of the sera were positive but, at day seven, 18% of the women had detectable pituitary antibodies. Furthermore, six to 12 months after delivery, 25% of the women who originally had autoantibodies showed clinical signs of pituitary deficiency, compared with 7% in the group which initially was negative for these specificities. However, since the authors used a complement consumption test, it remains to be established whether these interesting results can be reproduced by IFL or other techniques.

In the same report, there was also the description of a woman who developed Sheehan's syndrome and, when she was tested five years after delivery, her serum had pituitary autoantibodies at high titres. Since this is the only such report in Sheehan's syndrome it is difficult to define precisely the role of autoimmunity in this pathological entity. It could be postulated that vascular necrosis initiates the liberation of autoantigens from the damaged cells, thus being responsible for triggering secondary autoimmune processes. On the other hand, it is also possible that some cases diagnosed as Sheehan's syndrome are, in fact, primarily the end results of an autoimmune hypophysitis.

Using the IFL technique, several laboratories have produced data on the prevalence of pituitary autoantibodies in selected groups of patients and in the normal population. Their frequency in normal controls is generally low.
Figure 1. Indirect immunofluorescence on cryostat section of human pituitary gland stained with a serum containing PRL-producing cell antibodies. The final demonstration of the specificity of the reaction was obtained using a double fluorochrome technique with the corresponding anti-hormone antiserum.

Figure 2. Indirect immunofluorescence on cryostat section of human pituitary gland stained with serum containing the 'multiple cell pattern'. This reaction is primarily found in recent onset type I diabetic patients and in their predisposed ICA-positive first-degree relatives.
Figure 1. Indirect immunofluorescence on cryostat section of human pituitary gland stained with a serum containing PRL-producing cell antibodies. The final demonstration of the specificity of the reaction was obtained using a double fluorochrome technique with the corresponding anti-hormone antiserum.

Figure 2. Indirect immunofluorescence on cryostat section of human pituitary gland stained with serum containing the 'multiple cell pattern'. This reaction is primarily found in recent onset type 1 diabetic patients and in their predisposed ICA-positive first-degree relatives.
We have not detected these specificities in 48 normal sera (Mirakian et al., 1982), but when Pouplard et al. (1983) screened a population of normal elderly people, three out of 21 individuals were positive.

Initially, pituitary antibodies were not detected in patients with idiopathic panhypopituitarism (Bottazzo et al., 1975), but subsequently they were found in 10 out of 21 sera from similar patients using rat and human pituitary sections (Pouplard et al., 1980b). However, about 15% of sera from patients with mild hypopituitarism had autoantibodies to PRL cells, but investigation of sera from 21 patients with isolated ACTH, TSH or gonadotrophin selective deficiencies revealed positive antibodies in only one case and they were against unidentified pituitary cells (Table 2). This low prevalence was not confirmed in another study which showed that about half of the cases

<table>
<thead>
<tr>
<th>Type of deficiency</th>
<th>Number of patients tested</th>
<th>Positive immunofluorescence on human pituitary</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PRL-producing cells</td>
</tr>
<tr>
<td>Panhypopituitarism</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Mild hypopituitarism</td>
<td>41</td>
<td>7 (17%)</td>
</tr>
<tr>
<td>Isolated TSH/ACTH/LH/FSH</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Hypogonad infertility</td>
<td>54</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>138</td>
<td>9</td>
</tr>
</tbody>
</table>

with isolated pituitary deficiencies had pituitary antibodies (Pouplard et al., 1980a). Again the use of different substrates might account for these discrepancies.

Until now, only one documented case of GH deficiency had been reported with autoantibodies which selectively stained human GH-producing cells. Addison's disease and thyroiditis were recorded in the patient's family members (Bottazzo et al., 1980b). After this initial description, an extensive search was undertaken in children with growth defects, and in this selected group of more than 200 sera more than 5% reacted with isolated cells in the anterior pituitary (Table 3). The majority proved to be against PRL cells,

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. tested</th>
<th>Positive IFL on human pituitary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PRL-producing cells</td>
</tr>
<tr>
<td>Turner's syndrome (XO or mosaics)</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>Down's syndrome</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>Idiopathic short stature</td>
<td>397</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>483</td>
<td>7</td>
</tr>
</tbody>
</table>

6%
three sera reacted with GH cells and few remained to be identified. Pituitary antibodies in patients with chromosomal abnormalities and known to have growth retardation are rare.

Unexpectedly, when antibodies were sought in histologically proven cases of lymphocytic hypophysitis, only one positive reaction against pituitary cells was detected (Mayfield et al., 1980), but, as mentioned previously, only a few patients with this lesion have been screened so far. It can be speculated that, at the time when the histological observation was made, the autoimmune process was already partially burned out and pre-existing circulating antibodies had declined to an undetectable level. This might also explain why immunoglobulins bound to pituitary cells were not seen in one of the cases with florid lymphocytic hypophysitis studied close to the time of diagnosis by a direct IFL technique (Asa et al., 1981).

PRL-producing cell antibodies are found in about 10% of patients with endocrine autoimmune diseases (reviewed by Bottazzo et al., 1980c). Clinically, these patients had no apparent signs of overt pituitary defects and the thyrotropin releasing hormone test gave flat PRL responses in only two out of the eight cases investigated (Doniach et al., 1982). It was somewhat surprising to detect PRL-producing cell antibodies in a high proportion of patients with idiopathic hypoparathyroidism and in those with Candida infection (Table 4). It is known that these two conditions are often associated, but specific antibodies to parathyroid chief cell antibodies are rare and many laboratories still have difficulties in reproducing their original description (reviewed in Bottazzo et al., 1986b and Chapters 4 and 6). Clearly more studies are needed to fully establish the clinical and pathogenetic relevance of pituitary antibodies in idiopathic parathyroid deficiency cases, strongly suspected to have an autoimmune origin.

After investigating 23 patients with idiopathic Addison's disease, Ludwig and Scherntaner (1978) found antibodies against PRL-producing cells in two cases. A postmortem examination was carried out in one of them, and the anterior pituitary gland showed a dense focal lymphocytic and plasma cell infiltration. The patient was a woman with normal adenohypophysis function but, interestingly, immunoglobulin deposition was selectively detected by direct IFL in still-functioning PRL-producing cells, identified by

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. tested</th>
<th>PRL-producing cells</th>
<th>GH-producing cells</th>
<th>Unidentified cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyendocrine syndromes</td>
<td>217</td>
<td>25</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Idiopathic hypoparathyroidism</td>
<td>53</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>24</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other autoimmune disease*</td>
<td>391</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>685</td>
<td>52</td>
<td>1</td>
<td>2</td>
</tr>
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</table>

8%

* Includes type I diabetes, myasthenia gravis, thyroid disease, fundal atrophic gastritis, Addison’s disease, vitiligo and alopecia.
the corresponding anti-hormone antiserum. Occasional lymphocytic infiltration has previously been described at autopsy in patients with Addison’s disease but this was the first case where circulating antibodies to PRL cells were detected before the histological confirmation of hypophysitis. Thus, it appears that pituitary autoantibodies may have a potential pathogenetic role, at least in the early phases of tissue damage.

Other populations of patients have been screened for the presence of pituitary antibodies. In a group of patients with Alzheimer’s disease, Pouplard et al. (1983) found that 96% of sera reacted with PRL-producing cells. However, these results were obtained using human pituitaries collected at postmortem and we were unable to reproduce these potentially interesting data when the test was performed on fresh human or baboon tissues. In our hands, the frequency of anti-PRL-producing cell antibodies in 20 patients with Alzheimer’s disease was not dissimilar from that found in our normal control population (Philpot et al., 1985).

In a separate study (Pouplard, 1984), 25 out of 52 sera from children with cryptorchidism had antibodies against luteinizing hormone (LH) and follicle-stimulating hormone (FSH)-producing cells using guinea pig pituitaries. Again, because of the use of animal substrate the interpretation of these results is questionable, although the observation is of potential interest.

Antibodies giving the ‘multiple’ IFL pattern on anterior pituitary have unexpectedly been described in 17% of newly diagnosed insulin-dependent diabetic patients and, in addition, in a proportion of their first-degree relatives. The test was carried out using fresh human pituitary tissue as a substrate (Mirakian et al., 1982). These antibodies were of low titre, predominantly of IgG and IgM classes, and half of them fixed complement. The presence of pituitary antibodies in the unaffected relatives was closely associated with ICA and four out of seven relatives who initially possessed these specificities subsequently developed overt diabetes. This type of pituitary antibody tended to persist during the prediabetic period, but they decreased in titre after diagnosis.

Their role in the pathogenesis of type I diabetes is still unknown and does not appear to be associated with hypophysitis, since there is no direct evidence of impaired pituitary function in newly diagnosed diabetic children. Interestingly, however, identical twins showed growth delay in the period before diabetes was diagnosed, with a mean equivalent to 35 weeks (Hoskins et al., 1985). It is our impression that, in general, the pituitary has been underestimated as a potential ‘contributor’ to the process leading to β cell destruction. Its role in the long prediabetic period which precedes the onset of clinical symptoms (reviewed by Bottazzo et al., 1986a) could be much more relevant than previously anticipated. In hypothetical terms it can be envisaged that fluctuating destructive immune phenomena directed selectively against pituitary cells could eventually lead to short-lasting hyposecretion of certain pituitary hormones. On the other hand, the possibility also exists that stimulatory immune factors (? autoantibodies) could exert a hypersecretory action at the level of the gland with consequent increase of circulating pituitary hormones, which in turn might contribute to β cell regeneration (Bottazzo, 1984).
Finally, autoantibodies against pituitary cells have been found in patients treated with pituitary implants of iridium (Etzrodt et al., 1984). Of 55 patients tested, 11 showed reactivity against a variety of pituitary cells. It is interesting to recall that there was no correlation in this study between the pituitary dysfunction diagnosed in the patients and the presence of autoantibodies in their serum against selective pituitary cells populations.

PITUITARY CELL SURFACE AUTOANTIBODIES

To be visible to the immune-competent cells, and to be susceptible to the attack by circulating autoantibodies and complement, an autoantigen has to be expressed on the surface of the target cells. In endocrine autoimmunity this has been conclusively demonstrated for the thyroid microsomal (Khoury et al., 1981b) and the adrenal (Khoury et al., 1981a) antigen, but in other organ-specific systems, such as surface antibody to pancreatic β cells, the question still remains unresolved (reviewed by Bottazzo et al., 1987). Therefore, it was logical to examine viable pituitary cells in primary culture for the possible presence of surface autoantigens recognizable by patients' autoantibodies. However, for technical reasons, primarily inherent in the scarcity of normal human pituitary cells, this particular approach is still in its infancy.

Preliminary results have shown that sera which react with cytoplasmic antigen on tissue sections (e.g. PRL-producing cells) also stain viable pituitary cell monolayers, but the cell specificity of the surface reaction seems to be lost as the antibodies bind indiscriminately to the majority of pituitary cells present in the culture (R. Mirakian, unpublished data). The final interpretation of these results remains uncertain and clearly better refinements of the culture technique are needed before one can draw any definitive conclusions about the real pathogenetic significance of surface-reactive pituitary antibodies.

CELL-MEDIATED IMMUNE STUDIES IN HUMANS

In one study, a lymphocyte proliferation assay was used to assess the response to a crude extract of human pituitary in nine normal subjects and three children with idiopathic hypopituitarism. Only one of the patients had lymphocytes strongly responsive to pituitary antigens. The reaction was specific and, interestingly, the child also had alopecia and hypogammaglobulinaemia (Sobel et al., 1984). These data support the concept that an autoimmune mechanism directly mediated by T cells could be responsible for the attack to pituitary cells. However, autoantibodies were not measured in this young patient and clearly this work has to be extended to a larger number of cases and better purified pituitary antigens have to be used to further define the characteristic of the reaction.
ANIMAL MODELS

The first animal model of autoimmune hypophysitis was successfully obtained by Levine in 1967. Homogenates of pituitary glands prepared from both the anterior lobe and the whole pituitary gland were injected into rats in Freund's adjuvant. Histologically, hypophysitis was confirmed, but there was no clinical sign of pituitary dysfunction in the treated animals. The anterior lobe taken 13–20 days after immunization showed diffuse infiltration by mononuclear cells and some necrosis of the parenchymal cells. The posterior and intermediate lobes had only minimal inflammation. As mentioned, there was an increase in the severity of the pituitary inflammation in animals during the postpartum period which reflects the predilection of the human lymphocytic hypophysitis to occur in pregnant women or after delivery. Moreover, there was no inflammation detected in thyroid gland or in the pancreas of these animals, confirming the organ specificity of the autoimmune attack. In 1982, Klein et al obtained evidence for cellular mediated immunity in rabbits immunized with homologous pituitary homogenates in complete Freund's adjuvant. Lymphocytic infiltration was present in five out of the seven treated animals and pituitary extracts significantly stimulated lymphocytic proliferation in four of the five animals. However, circulating pituitary autoantibodies were not observed and there was no functional impairment of the pituitary.

Another experimental model was described by Onodera et al (1981). Inbred mice were infected with a reovirus type I. Development of a transient diabetes and retarded growth occurred in these animals as a consequence of the infection. The authors showed the presence of viral particles within the islet cells in the pancreas and in the GH-producing cells of the pituitary gland. Inflammatory cells were found in the islets of Langerhans and in the anterior but not in the posterior part of the gland. Cross-reacting antibodies were produced in these animals and they stained islets, anterior pituitary, gastric parietal cells and thyroid glands, and were directed against GH and a 35 kDa protein commonly present in all these cells (proved by absorption and precipitation studies respectively). This is in striking contrast with human autoantibodies which are strictly cell- or organ-specific, uniquely directed against distinct cellular components and do not react with the hormone itself (reviewed by Bottazzo et al, 1986a).

MAJOR HISTOCOMPATIBILITY (MHC) MOLECULE EXPRESSION ON PITUITARY CELLS

In several tissues affected by autoimmunity, an 'inappropriate' expression of MHC Class II molecules has been described. This is the case with thyrocytes from patients with autoimmune thyroid disease or with β cells in patients with type I diabetes mellitus (discussed in Chapter 1). Class II expression on thyrocytes can be induced by several modulators, including interferon-γ (IFN-γ) (Todd et al, 1985), whereas pancreatic islet cells are much more resistant. Expression of Class II products on these cells, in fact, could only
be attained when isolated human islets were incubated with a combination of IFN-\(\gamma\) and tumour necrosis factor (Pujol-Borrell et al., 1987). All these data indicate the importance of Class II expression in the induction of perpetuation of autoimmunity (Bottazzo et al., 1983) and it is recognized now as an important common feature in organ-specific autoimmunity (reviewed by Bottazzo et al., 1986).

Following these lines, we performed preliminary experiments using collagenase-digested pituitary cells from two patients affected by Cushing’s disease and two from acromegaly. Primary cultures were established on coverslips and spontaneous MHC Class I and Class II products were investigated by means of an IFL technique and appropriate monoclonal antibodies, both on the cell surface and in the cytoplasm. MHC Class I molecules were always detected in the cytoplasm of these cells, but they were also expressed on the plasma membrane of viable cell preparations. On the contrary, no MHC Class II molecules were observed in similarly treated pituitary cell cultures. However, MHC Class II products could be induced in pituitary cell preparations after addition of IFN-\(\gamma\) at a dose of 500 U/ml. This was observed in monolayers prepared from a GH-producing adenoma (R. Mirakian, personal communication).

The fact that pituitary cells are able to synthesize Class II molecules in experimental conditions indicates that the same phenomenon may occur in vivo, thus making these cells ideally placed to interact with activated immune-competent cells. Obviously, it is vital to extend these ‘in vitro’ observations by an extensive analysis of the available pituitaries from patients with lymphocytic hypophysitis, by confirming the presence of MHC molecule expression also in the affected tissue, trying to correlate this phenomenon with immunoglobulin and complement depositions and characterizing the type of lymphocytes infiltrating the gland. It remains to be established why the posterior pituitary, which has different embryological origin and contains cells of the neuronal type, appears, in general, to be disassociated from the florid autoimmune process almost exclusively affecting the anterior part of the gland. This is confirmed by the rarity of detection of concomitant defects of antidiuretic hormone secretion in association with selective anterior pituitary hormone deficiencies.

SUMMARY

Pituitary autoimmunity is now a well-documented pathological entity. An increasing number of cases have been diagnosed thanks to improved immunological and clinical assessment. Hypopituitarism occurs in pregnancy and in the postpartum period or is often associated with other autoimmune diseases. The diagnostic and predictive value of pituitary autoantibodies is at present unclear. There is no strict correlation between the presence of these specificities and the corresponding pituitary hormone deficiencies, possibly because of the long latency period between the onset of an autoimmune recognition and clinically detectable pituitary dysfunctions. Clearly, more studies have to be carried out in patients with
incipient lymphocytic hypophysitis to find suitable tests which will enable
identification of reliable markers (both humoral and cell mediated) for
predicting the subsequent onset of the disease. However, there is no doubt
that the pituitary gland has to be regarded as another endocrine organ prone
to an autoimmune attack. Our work in the future should concentrate on fully
characterizing autoimmune phenomena in situ and finally proving that
inappropriate MHC Class I and II expression is also detectable in this target
organ. Finally, it is strongly advisable to plan studies which look at the
possible HLA association in proved diagnosed cases, its linkage in family
members and for direct involvement of autoantibodies and cell immune
reactions in mediating recognition and ultimate destruction of pituitary
cells.

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AUTOIMMUNITY AND THE PITUITARY


