Cytosolic Autoantigens in Lymphocytic Hypophysitis*

PATRICIA A. CROCK

Department of Pediatric Endocrinology and Diabetes, John Hunter Children’s Hospital, Newcastle 2310, New South Wales, Australia

ABSTRACT

Lymphocytic hypophysitis was first recognized postmortem, then by biopsy, but detection of antipituitary autoantibodies by immunofluorescence has proved unsatisfactory. Immunoblotting has the dual advantages of increased specificity and identification of the mol wt of autoantigens.

Sera from 115 patients and 52 normal subjects were immunoblotted against human autopsy pituitary cytosolic proteins. Among the neurosurgical cohort (30), 10 patients had biopsy-proven lymphocytic hypophysitis, and 20 had hypopituitarism secondary to tumor. There were 22 cases with suspected hypophysitis; 47 with either Hashimoto’s, Graves’, or Addison’s diseases; and 15 with rheumatoid arthritis.

Antipituitary autoantibodies reactive to a 49-kDa pituitary cytosolic protein were found in 70% of biopsy-proven lymphocytic hypophysitis, 55% of suspected hypophysitis, 42% of Addison’s disease, 20% of pituitary tumors, 15% of patients with thyroid autoimmunity, 13% of rheumatoid arthritis patients, and 9.8% of normal subjects. Reactivity to a 40-kDa cytosolic protein was also found in 50% of patients with biopsy-proven disease. These 49- and 40-kDa autoantigens are conserved across species and are not exclusive to pituitary tissue.

Immunoblotting has demonstrated antipituitary autoantibodies to 49- and 40-kDa cytosolic proteins in biopsy-proven cases of lymphocytic hypophysitis. (J Clin Endocrinol Metab 83: 609–618, 1998)

Lymphocytic hypophysitis is considered an autoimmune reaction in the anterior pituitary (1–3). The classical presentation is peripartum hypopituitarism, often with a pituitary mass and visual failure (3–8). Secondary adrenal insufficiency is an almost universal feature, which, when undiagnosed, has proven fatal (9, 10). In the early stage, the pituitary gland is enlarged like a pituitary tumor (11–13), from which it cannot be distinguished on computed tomography or magnetic resonance imaging (MRI) scanning (11, 14). In the later stages, the gland may atrophy, leaving an empty sella (15), as occurs in Sheehan’s syndrome. Spontaneous resolution of both the mass (16, 17) and the hypopituitarism (18–21) have been reported. Neurosurgical intervention has led to irreversible pituitary failure in some cases (6, 22).

Although the literature records 101 biopsy-proven cases, the relevant target autoantigens have not been identified previously. A serological test for the detection of antipituitary autoantibodies would confirm the autoimmune nature of this disease and help to determine its prevalence and define the clinical spectrum. The need for neurosurgery, with its attendant risks, may be reduced. An immunoblotting test has been developed for the detection of antipituitary autoantibodies (23). We have now used this to identify at least two target autoantigens in lymphocytic hypophysitis.

Materials and Methods

Patient and normal control sera

Serum samples were obtained from sources in Australia, Canada, and the USA. Sera from 10 patients (8 women and 2 men; mean age, 38.5 yr) with biopsy-proven lymphocytic hypophysitis and 22 patients [15 women (mean age, 34 yr) and 7 men (mean age, 50 yr)] with suspected disease were studied. The criteria for suspecting hypophysitis included the diagnosis of isolated ACTH deficiency or the presence of hypopituitarism in a patient with autoimmune disease or during the peripartum period. Patients with diabetes insipidus were tested for sarcoidosis by angiotensin-converting enzyme levels and were negative. Clinical details are outlined in Tables 1-3. The histology from all the biopsy-proven patients was reviewed by one neuropathologist, Dr. R. McD. Anderson, who confirmed the diagnosis of lymphocytic hypophysitis.

Sera from 20 patients with hypopituitarism secondary to irradiation or pituitary adenoma, from 47 patients with organ-specific autoimmune diseases (Hashimoto’s thyroiditis, n = 21; Graves’ disease, n = 12; Addison’s disease, n = 14), and from 15 patients with rheumatoid arthritis were also assayed for antipituitary autoantibodies. Sera were collected from 32 normal subjects (32 women and 20 men; age range, 19–60 yr; mean, 29.0 yr) who were laboratory and general staff attending the Staff Clinic at the Alfred Hospital (Melbourne, Australia) for routine posthepatitis B vaccine serology. Exclusion criteria included any major illness or an autoimmune disease. Sera from all patients with hypophysitis and from 27 normal subjects (age range, 20–60 yr; mean, 33.2 yr) were also assayed for other autoantibodies.

Ethics approval for the study was obtained from the Alfred Hospital ethics committee, and informed consent was given before the collection of blood samples.

Detection of antipituitary autoantibodies by immunoblotting

Normal human autopsy pituitary tissue was homogenized in phosphate-buffered saline with protease inhibitors (aprotinin, leupeptin, pepstatin, phenylmethylsulfonylfluoride, and ethylendiamine tetracetae) and centrifuged at 400 × g and then at 100,000 × g to give cytosolic and membrane fractions. Pituitary cytosol preparations were fractionated on SDS-polyacrylamide gels by electrophoresis under reducing conditions. The total protein loaded was constant at 50 μg/well. Monkey, rat, and ovine pituitary tissues and the mouse ACTH-secreting AtT20 cell line were handled in the same manner. Separated proteins were transferred to Immobilon (Bio-Rad, Hercules, CA) polyvinylidene difluoride (PVDF) membranes and incubated with experimental or control serum diluted 1:50 in 1% BLOTTO-phosphate-buffered saline overnight at 4 C. Reactivity to pituitary proteins was detected using alkaline phosphatase-conjugated goat antihuman IgG antiserum (Bio-Rad, Richmond, CA) and a color reaction with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium. Autoantibody...
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Past history</th>
<th>Presenting symptoms</th>
<th>CT/MRI</th>
<th>Deficits</th>
<th>Progress</th>
<th>Autoimmune disease</th>
<th>49-kDa antibody reactivity</th>
<th>40-kDa antibody reactivity</th>
<th>Time from biopsy to pituitary antibody test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57</td>
<td>F</td>
<td>Rheumatic heart disease</td>
<td>Collapse due to pituitary apoplexy</td>
<td>Mass SSE</td>
<td>Panhypopituitarism</td>
<td>Hypophysectomy, permanent hypopituitarism</td>
<td>ANF 1:200</td>
<td>+</td>
<td>+</td>
<td>17 months</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td>F</td>
<td>G1P1, psoriasis</td>
<td>2º amenorrhea</td>
<td>Mass 3.5 cm</td>
<td>ACTH, TSH, LH/FSH</td>
<td>Biopsy</td>
<td>IDDM (7 yr)</td>
<td>+</td>
<td>+</td>
<td>4.5 yr</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
<td>M</td>
<td>Panhypopituitary, 12–18 months</td>
<td>Mass</td>
<td>Panhypopituitarism</td>
<td>Hypophysectomy</td>
<td></td>
<td>Nil</td>
<td>+</td>
<td>+</td>
<td>16 months</td>
</tr>
<tr>
<td>4 (Ref. 8)</td>
<td>29</td>
<td>F</td>
<td>G1P1</td>
<td>Rapid visual failure 25/40, gestation, H/A</td>
<td>Mass, SSE</td>
<td>ACTH, GH, TSH, LH/FSH</td>
<td>Biopsy, menses returned</td>
<td></td>
<td>-</td>
<td>+</td>
<td>16 months</td>
</tr>
<tr>
<td>5 (Ref. 24)</td>
<td>34</td>
<td>F</td>
<td>G4 P4, 10 yr postpartum nephrogenic diabetes insipidus (lithium)</td>
<td>Fatigue, blurred vision, headache, 2º amenorrhea</td>
<td>Mass, SSE</td>
<td>ACTH, LH/FSH, PRL 4× N</td>
<td>Failed trial of bromocriptine, hypophysectomy, hypopituitarism</td>
<td>ANF 1:400</td>
<td>+</td>
<td>-</td>
<td>3 months</td>
</tr>
<tr>
<td>6 (Ref. 7)</td>
<td>21</td>
<td>F</td>
<td>G1P1</td>
<td>Rapid visual failure 28/40 gestation, H/A</td>
<td>Mass, SSE</td>
<td>PRL 7× N</td>
<td>Failed trial of steroids, hypophysectomy, hypopituitarism</td>
<td></td>
<td>-</td>
<td>-</td>
<td>7 yr</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>F</td>
<td>G3P1</td>
<td>Third trimester H/A, bitemporal, hemianopia</td>
<td>Mass, SSE</td>
<td>ACTH, TSH</td>
<td>Hypophysectomy, hypopituitarism</td>
<td></td>
<td>-</td>
<td>-</td>
<td>15 months</td>
</tr>
<tr>
<td>8</td>
<td>52</td>
<td>F</td>
<td>Facioscapulohumeral dystrophy</td>
<td>6 months visual disturbance</td>
<td>Mass, SSE</td>
<td>Panhypopituitarism</td>
<td>Hypophysectomy, hypopituitarism</td>
<td>IDDM, pernicious anemia</td>
<td>+</td>
<td>-</td>
<td>5 yr</td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td>F</td>
<td>Meningoencephalitis</td>
<td>Mass, contrast ring</td>
<td>6-mm mass in stalk</td>
<td>LH/FSH, TSH, diabetes insipidus</td>
<td>Hypophysectomy, hypopituitarism</td>
<td>ANF 1:200</td>
<td></td>
<td></td>
<td>12 months</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>M</td>
<td>Ischemic heart disease</td>
<td>Persistent fevers 12 months of diabetes insipidus, hypogonadism, hypothyroidism</td>
<td></td>
<td></td>
<td>Hypophysectomy, panhypopituitarism</td>
<td></td>
<td>+</td>
<td>-</td>
<td>18 months</td>
</tr>
</tbody>
</table>

CT, Computed tomography; MRI, magnetic resonance imaging; SSE, Suprasellar extension; G, gravida; P, para; N, normal; HA, headache; ANF, antinuclear factor; IDDM, insulin-dependent diabetes mellitus.
titers were also performed at serum dilutions of 1:100, 1:200, 1:500 and 1:1,000. For further details, see Crock et al.

Species specificity
Positive sera were tested by immunoblotting on pituitary tissue from cynomologous monkeys, sheep, rats, and murine AtT20 cells. Fresh-frozen cynomologous monkey pituitary glands were obtained from the Commonwealth Serum Laboratories (Melbourne, Australia) with ethics and quarantine approvals. Ovine pituitary tissue was obtained from an abattoir. Rat pituitaries came from laboratory animals killed for other purposes. All tissues were prepared as outlined above for human tissue.

Tissue specificity
Positive sera, as identified above, were tested by immunoblotting against cytosolic preparations from fresh-frozen cynomologous monkey brain, adrenal, thyroid, liver, spleen, serum, and skeletal muscle.

Screening for other autoantibodies
Sera from all patients with hypophysitis and from 27 of the 52 normal subjects were tested for a wide range of autoantibodies, looking for both organ-specific and nonorgan-specific autoimmunity. These included autoantibodies to thyroid, stomach, ovary, adrenal, kidney, liver, smooth muscle, nuclei, and mitochondria. The 25 normal subjects not tested were younger (mean age, 25.8 yr). Sera were screened for antithyroid microsomal and antithyroglobulin autoantibodies, using Thymune*-M and Thymune*-T kits, respectively (Murex Diagnostics, Temple Mill, UK). Antigastric parietal cell, antismooth muscle, and antimitochondrial autoantibodies were detected by indirect immunofluorescence on fresh-frozen sections of mouse stomach, kidney, and liver. Antinuclear antibodies were detected by indirect immunofluorescence on Hep-2 cells, and antiovarian and antiadrenal autoantibodies were detected by indirect immunofluorescence on fresh-frozen sections of cynomologous monkey tissue.

Statistical analysis
Reactivity to the 49- or 40-kDa cytosolic proteins by immunoblotting was compared between groups using Fisher’s exact test. \( P < 0.05 \) was considered statistically significant.

Results

Antipituitary autoantibodies
Autoantibodies against a 49-kDa pituitary cytosolic protein were demonstrated by immunoblotting in the sera of 7

### TABLE 2. Suspected lymphocytic hypophysitis: female patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Past history</th>
<th>Presenting symptoms</th>
<th>CT/MRI</th>
<th>Hormone deficits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>F</td>
<td>G2P2</td>
<td>Headaches, postpill amenorrhea, 2nd infertility</td>
<td>Mass SSE, contrast ring</td>
<td>LH/FSH, normal vision</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>F</td>
<td>Pill-induced hepatic adenomata, G2P1</td>
<td>Severe headache, hypoglycemic coma in 3rd trimester</td>
<td>N postpartum</td>
<td>ACTH, GH, transient diabetes insipidus, normal vision</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>F</td>
<td>Faco-seapulo-humeral dystrophy, Guillain-Barre syndrome, G2P1</td>
<td>Headache, vomiting, recurrent hypoglycemic coma in 1st and 2nd trimesters</td>
<td>Mass, 5 mm</td>
<td>Panhypopituitarism, normal vision</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>F</td>
<td>G2P2</td>
<td>Fatigue, anorexia, hypoglycemic coma in 3rd trimester, 29/40</td>
<td>N postpartum</td>
<td>ACTH, LH/FSH, PRL, TSH</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>F</td>
<td>G0</td>
<td>2nd amenorrhea, galactorrhea</td>
<td>PRL 7× N, LH/FSH, TSH</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>F</td>
<td>Diabetes insipidus for 9 yr</td>
<td>2nd amenorrhea</td>
<td>N</td>
<td>LH/FSH, diabetes insipidus</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>F</td>
<td>G2P2, alopecia</td>
<td>Failure to lactate 9 weeks postpartum</td>
<td>N</td>
<td>ACTH, TSH</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>F</td>
<td>G2P2</td>
<td>Hypoadrenalism, isolated ACTH deficiency</td>
<td>N</td>
<td>ACTH, TSH, PRL</td>
</tr>
<tr>
<td>9</td>
<td>35</td>
<td>F</td>
<td>G2P1</td>
<td>Heads in 2nd and 3rd trimesters, failure to lactate postpartum</td>
<td>Mass</td>
<td>Panhypopituitarism</td>
</tr>
<tr>
<td>10</td>
<td>32</td>
<td>F</td>
<td>G1P1</td>
<td>Failure to lactate postpartum, transient visual field defect 10 days postpartum</td>
<td>N</td>
<td>ACTH, LH/FSH, PRL</td>
</tr>
<tr>
<td>11</td>
<td>24</td>
<td>F</td>
<td>G1P1</td>
<td>Secondary amenorrhea 2 yr postpartum</td>
<td>N</td>
<td>ACTH, GH, PRL 2× N</td>
</tr>
<tr>
<td>12</td>
<td>42</td>
<td>F</td>
<td>G2P2</td>
<td>Hypoadrenalism</td>
<td>N</td>
<td>Isolated ACTH deficiency</td>
</tr>
<tr>
<td>13 (Ref. 44)</td>
<td>30</td>
<td>F</td>
<td>G3P3</td>
<td>3rd trimester fatigue, wt loss, anorexia, amenorrhea, and failure to lactate postpartum</td>
<td>N</td>
<td>Transient T₄ increase and hypercalcemia, panhypopituitarism</td>
</tr>
<tr>
<td>14</td>
<td>43</td>
<td>F</td>
<td>G3P3</td>
<td>Fatigue</td>
<td>Thick stalk</td>
<td>Isolated ACTH deficiency</td>
</tr>
<tr>
<td>15</td>
<td>68</td>
<td>F</td>
<td>G2P2</td>
<td>Marked fatigue, weight loss</td>
<td>N</td>
<td>Isolated ACTH deficiency</td>
</tr>
</tbody>
</table>

Downloaded from jcim.endojournals.org at Welch Medical Library - JHU Serials Department on April 10, 2007
of 10 patients with biopsy-proven lymphocytic hypophysitis (by Fisher’s exact test, \( P < 0.0001 \) vs. controls) and in 12 of 22 patients who were suspected of having the disease (by Fisher’s exact test, \( P < 0.0001 \) vs. controls) (see Fig. 1). Six of the 19 patients with antipituitary autoantibodies had a titer of more than 1:1000 (see Tables 1–4 and Fig. 2); of whom 3 had pituitary apoplexy and 2 had isolated ACTH deficiency (1 with an empty sella). Five of 10 biopsied patients and 6 suspected patients also had autoantibodies to a 40-kDa pituitary cytosolic protein (see Tables 1–3; by Fisher’s exact test, \( P = 0.005 \) and \( P = 0.06 \), respectively, vs. controls).

Sera from 5 of 52 control subjects were reactive to the 49-kDa cytosolic protein (by Fisher’s exact test, \( P = 0.0001 \) vs. both biopsy-proven and suspected patients), but none had a titer of more than 1:200 (see Fig. 1 and Table 4). Four normal control subjects had autoantibodies to the 40-kDa cytosolic protein, of whom one had a titer of more than 1:1000.

Autoantibodies to a 49-kDa protein were detected in 6 of 14 Addison’s patients (by Fisher’s exact test, \( P = 0.18 \) vs. biopsy-proven hypophysitis), 1 of 12 Graves’ disease patients (\( P < 0.005 \) vs. biopsy), 4 of 21 Hashimoto’s disease patients (\( P < 0.01 \) vs. biopsy), 2 of 15 rheumatoid arthritis patients (\( P < 0.01 \) vs. biopsy), and 4 of 20 patients with pituitary tumours (\( P = 0.01 \) vs. biopsy; see Table 4). Only 3 of these 62 patients had a titer of more than 1:1000: 1 each with Addison’s disease, rheumatoid arthritis, and a postoperative pituitary tumor.

Other polypeptides were detected by individual sera (e.g. an 88-kDa band was seen in two patients, one illustrated in Fig. 1, lane 4), but because these were not consistently present in hypophysitis cases, they will not be further considered here. Immunoblotting is known to detect multiple autoantigens (26). Nonspecific binding was seen in all experiments at approximately 25, 50, and 64 kDa with second antibody alone and was reduced by depleting the pituitary cytosolic fraction of IgG. It was not seen when...
TABLE 3. Suspected lymphocytic hypophysitis: male patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Past history</th>
<th>Presenting symptoms</th>
<th>CT/MRI</th>
<th>Hormone deficits</th>
<th>Progress</th>
<th>Autoimmune disease</th>
<th>49-kDa antibody reactive</th>
<th>49-kDa antibody reactive</th>
<th>Other autoantibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49</td>
<td>M</td>
<td></td>
<td>6-month wt loss, malaise, myalgia, anorexia, nausea, vomiting</td>
<td>Partial empty sella</td>
<td>Isolated ACTH deficiency</td>
<td>Well on replacement therapy</td>
<td>+</td>
<td>&gt;1:1,000</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2 (Ref. 25)</td>
<td>44</td>
<td>M</td>
<td></td>
<td>6 months wt loss, fatigue, arthralgia, gynecomastia</td>
<td>N</td>
<td>Isolated ACTH deficiency</td>
<td>Prednisone (7.5 mg), 9-kg wt gain, normotensive</td>
<td>+</td>
<td>&gt;1:1,000</td>
<td>–</td>
<td>Antinuclear antibodies 1:320 (nucleolar)</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>M</td>
<td></td>
<td>Hypoglycemic comas, 12-kg wt loss, cramps, headaches, hypogonadism</td>
<td>N</td>
<td>Isolated ACTH deficiency</td>
<td>Well on cortisone acetate (37.5 mg daily)</td>
<td>–</td>
<td>–</td>
<td>Weak</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>M</td>
<td>Blind right eye (war injury)</td>
<td>Mass SSE, hypogonadism</td>
<td>N</td>
<td>Panhypopituitarism, diabetes insipidus, normal left eye</td>
<td>10 months later: CT normal diabetes insipidus and hypopituitarism both resolved</td>
<td>Nil</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>M</td>
<td></td>
<td>Diabetes insipidus</td>
<td>N</td>
<td>Diabetes insipidus</td>
<td>Permanent diabetes insipidus Iritis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>M</td>
<td>Deep venous thrombosis</td>
<td>Idiopathic hypopituitarism</td>
<td>N</td>
<td>ACTH, GH, LH/FSH</td>
<td>Panhypopituitarism</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>59</td>
<td>M</td>
<td>Hypertension</td>
<td>Acromegaloïd, hypogonadism, bitemporal hemianopia</td>
<td>N</td>
<td>GH, LH/FSH, normal IGF-I</td>
<td>Hypogonadal Diabetes mellitus</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

SSE, suprasellar extension; CT, computed tomography; MRI, magnetic resonance imaging; N, normal.
animal tissues were used. Human Igs from blood present in the pituitary glands at homogenization are the most likely explanation.

Other autoantibodies

One or more autoimmune diseases were present in 5 patients with biopsy-proven lymphocytic hypophysitis: type I diabetes, Graves’ disease, pernicious anemia, and positive antinuclear factor (Table 1). Sixteen of 22 patients with suspected disease also had 1 or more autoimmune diseases, as outlined in Tables 2 and 3. Seven control sera had positive antinuclear factor antibodies, with titers ranging from 1:200 to 1:1600 (normal range, 0–1:100), but were negative for all other antibodies. Low titer autoantibodies are often found in normal control subjects and are thought to represent natural antibodies.

Species specificity

Patient sera reactive to the 49-kDa cytosolic protein in human pituitary also reacted with a 49-kDa cytosolic protein in pituitary tissue from cynomologous monkey, sheep, and rat and the AtT20 cell line. Positive reactivity is shown in Fig. 3. Many other bands of reactivity are seen, particularly with nonhuman tissues, but these are shared with normal control sera. The 49-kDa band was particularly prominent in AtT20 cytosolic preparations, suggesting that this antigen may be highly expressed in these cells and their normal counterparts, the corticotroph (Fig. 3, lane 10). Thus, the 49-kDa autoantigen is conserved across many species and is not primate specific. The 40-kDa autoantigen was also present in monkey and rat brain (results not shown).

Tissue specificity

Patient sera reactive to the 49-kDa protein in human and monkey pituitary also reacted to a 49-kDa cytosolic protein in some other tissues, including monkey brain, thyroid, liver, and spleen, but not in skeletal muscle or serum (results not shown). Although the 49-kDa autoantigen is not pituitary specific, neither is it ubiquitous.

Discussion

Autoantibodies are the hallmark of autoimmunity, but they have been difficult to detect in pituitary autoimmune disease. Improved imaging of the pituitary by computed tomography scanning and MRI now displays more subtle pituitary masses, but still cannot distinguish a pituitary adenoma from lymphocytic hypophysitis (14). The diagnostic armamentarium needs to be expanded to include a reliable serological test. Our results may represent a first step in this direction.

The original report of antipituitary autoantibody activity described a complement fixation test and found reactivity in the serum of 18% of postpartum women, but gave no objective endocrine data to support their clinical relevance (27). Testing by immunofluorescence was developed in 1975 by Bottazzo et al., using fresh-frozen human pituitary tissue from hypophysectomies (28). The sera of 297 patients with polyglandular autoimmune disease were screened for anti-
pituitary autoantibodies on the premise that autoimmune diseases cluster. Nineteen patients had positive antipituitary autoantibodies to PRL-secreting cells, but again, none of these patients had evidence of pituitary insufficiency (28). Immunofluorescence has detected autoantibodies in only 2 of 7 patients with biopsy-proven lymphocytic hypophysitis (12, 29). These autoantibodies were believed to be of low titer and only specific to fresh-frozen pituitary glands of primate origin (28). Seventy-eight percent of reactions were only detected with undiluted or untitrated sera (28). However, other researchers have used pituitary tissue from guinea pig (30), rat (31), mouse AtT20 cells (31), pigs (32), and fetal tissue (33), with positive results in cryptorchidism (30), empty sella syndrome (34), isolated ACTH deficiency (31), Graves’ disease (32), and Cushing’s disease (33). A further problem with immunofluorescence has been the nonspecific binding of autoantibodies to corticotroph cells by Fc receptors (33, 35).

An alternative approach to the detection of antipituitary autoantibodies uses immunoblotting to overcome these problems (23). This method has been successful with autopsy pituitary glands as substrate. In the current study, we have detected antipituitary autoantibodies in patients with biopsy-proven and suspected autoimmune pituitary disease at titers of more than 1:1000. At this dilution the assay became more specific for lymphocytic hypophysitis, but did lose some sensitivity. We have identified at least two target autoantigens in pituitary autoimmune disease, which are cytosolic proteins of 49 and 40 kDa. The antigenic determinants recognized by these sera are conserved across species, including monkeys, sheep, rats, and mice. Conservation of autoantigenic epitopes across species is the general rule in autoimmunity.

We speculate that the 49-kDa autoantigen might be related to ACTH deficiency from corticotroph destruction. ACTH deficiency is the most prominent feature of lymphocytic hy-
Lymphocytic hypophysitis may be seen in isolation (9, 21). By contrast, ACTH is often the last hormone to be affected in patients with pituitary tumors. Two of three male patients with isolated ACTH deficiency in our series had high titer autoantibodies to the 49-kDa autoantigen. One had an empty sella on MRI scan, suggesting that his condition was the result of pituitary atrophy secondary to lymphocytic hypophysitis. The second patient has been shown by other investigators to have autoantibodies that bound to 200-nm secretory granules in rat corticotrophs (25). Isolated ACTH deficiency is more common in Japan, and an antipituitary autoantibody assay using immunofluorescence on AtT20 cells was reported to be positive in 70% of patients (31). When AtT20 cell cytosolic proteins were immunoblotted against our positive sera, strong reactivity to a 49-kDa protein was detected, and the target autoantigen appeared enriched. This suggests that the 49-kDa pituitary cytosolic protein is both present in corticotrophs and conserved across species. Reactivity to many other proteins in AtT20 cells was detected by sera from patients and normal subjects, highlighting the problems of nonspecific binding with nonprimate tissues and perhaps reflecting natural antibodies to xenoantigens.

Reactivity to the 49-kDa cytosolic protein was found in brain, adrenal, thyroid, liver, and spleen, but not in skeletal muscle or serum. The lack of pituitary specificity of this protein does not necessarily mitigate against its involvement with corticotrophs and ACTH deficiency. The precursor of ACTH, POMC, is processed in many cells in the body, except skeletal muscle (36), and target autoantigens are not always confined to the tissues primarily affected by the autoimmune process. For example, in primary biliary cirrhosis, the major autoantigen detected serologically is a ubiquitous mitochondrial enzyme, pyruvate dehydrogenase, but clinical disease is confined to the liver (37).

Immunoblotting methods often detect multiple autoantigens (26). It is now recognized that there are multiple target autoantigens in type I diabetes (38), Hashimoto’s thyroiditis, and Graves’ disease (39). Sera from 10 patients in our series reacted with a 40-kDa pituitary cytosolic protein, and this was particularly striking in the biopsy-proven cohort (50% positive). Some patients showed weak reactivity to multiple cytosolic proteins, which disappeared at greater serum dilutions. Some of this reactivity could be explained by the phenomenon of natural antibodies, which are usually in low titer and nonpathogenic. Strong reactivity to an 88-kDa protein, as shown in Fig. 1, lane 4, may represent another target autoantigen, as it was not seen with normal sera. Thus, at least two, if not three, pituitary autoantigens have been identified by our immunoblotting assay.

Unusual clinical presentations of lymphocytic hypophysitis in our series included two women with facio-scapulohumeral muscular dystrophy and diabetes mellitus, both of whom had high titer antipituitary autoantibodies (>1:1000) and one of whom had biopsy-proven disease. There were four cases with pituitary apoplexy, two during pregnancy, which has also not been reported previously in hypophysitis. Apoplexy was associated with high titer autoantibodies and in one case with ring enhancement of the pituitary mass on MRI. Although ring enhancement is seen with pituitary tumors, it has recently been reported as a feature of hypophysitis (14). It is also likely that pituitary apoplexy due to hypophysitis in the peripartum period can be misinterpreted as Sheehan’s syndrome (40). One male patient in our series had acromegaloism, and there are single reports of a coexistent GH-secreting adenoma (41) and elevated GH levels (42) with hypophysitis. One patient in our series presented with meningoencephalitis, and other cases have presented as lymphocytic meningitis (43). One woman presented with hypercalcemia associated with transient hyperthyroidism and secondary adrenal failure (44). Fifteen cases of hypophysitis have been reported in men (8, 45–52), and we add another seven male patients.

Diabetes insipidus occurs with lymphocytic hypophysitis (19, 43, 46, 47, 50), but rarely preoperatively in patients with pituitary adenoma. It was present in four patients in our series, three of whom had antipituitary autoantibodies. The term lymphohypophysitis (50) for patients with diabetes insipidus and lymphocytic infiltration of the pituitary stalk. Necrotizing infundibulo-hypophysitis has also been reported with diabetes insipidus (49). Perhaps these are part of the spectrum of lymphocytic hypophysitis.

A classical feature of lymphocytic hypophysitis is its association with pregnancy (1, 3, 4, 51–53). PRL levels may be high, simulating a prolactinoma (3, 54, 55), or deficient, causing lactation failure (4). Hyperprolactinemia was seen in four cases in our series. Lactational failure was seen in six patients, five of whom had antipituitary autoantibodies. This latter symptom is commonly seen in Sheehan’s syndrome, and we suspect that patients labeled as such but with no history of peripartum hemorrhage have unrecognized hypophysitis.

Four of 20 patients in our series, with known pituitary tumors, had antipituitary autoantibodies. Sera had been collected postoperatively and/or postradiotherapy. Radiation damage and tumors may induce autoantibody production (56), so these results may reflect the release of tissue antigens due to pituitary injury.

A range of other autoimmune diseases was present in 50% of our biopsy-proven patients, which is a little higher than the 30% reported by Cosman (4), but is in keeping with the tendency of autoimmune diseases to cluster. Autoimmune diseases were present in 60% of our suspected hypophysitis patients, but as hypopituitarism with autoimmune disease was often the rationale for requesting antipituitary autoantibodies, our series has this inherent selection bias. The incidence of antipituitary autoantibodies in patients with thyroid autoimmune disease was not significantly different from that in control subjects, suggesting that reactivity to the 49-kDa protein by immunoblotting is not a nonspecific finding in autoimmune disease.

Surprisingly, 42% of Addison’s sera had positive reactivity to a 49-kDa cytosolic protein, a protein also present in adrenal tissue. Patients with Addison’s disease have been found to have lymphocytes in the pituitary at autopsy (57), and patients with hypophysitis have had adrenal lymphocytic infiltration (58, 59). It is possible that the 49-kDa autoantigen is involved in POMC or ACTH processing (36). Alternatively, Addison’s sera may recognize a different autoantigen that coincidentally has the same molecular mass as the 49-kDa protein targeted in hypophysitis. This situation occurred in
type I diabetes, where the 64-kDa autoantigen was found to be both glutamic acid decarboxylase and BSA.

Identification of the 49- and 40-kDa cytotoxic autoantigens recognized by immunoblotting should help to elucidate the pathogenesis of lymphocytic hypophysitis.

Acknowledgments

Autopsy pituitary tissues were obtained from Dr. M. Chretien of the Institut de Recherche Clinique de Montreal, Canada. AT20 cells were obtained from Dr. I. Smith, Baker Institute (Melbourne, Australia). I am indebted to the following endocrinologists and physicians for sending sera; Dr. F. Alford, Dr. W. Braund, Dr. D. Chippis, Prof. D. Chisolm, Dr. P. Colman, Dr. D. Engler, Dr. M. Epstein, Dr. S. Hamblin, Prof. D. Healey, Dr. A. Hunter, Dr. M. McLean, Dr. A. McEilduff, Dr. G. Major, Dr. J. O’Day, Dr. P. Pullan, Prof. R. Smith, Dr. M. Sulway, Dr. J. Steil, Prof. B.-H. Toh, Dr. D. Topliss, and Dr. M. Zacharin; to Dr. John Downes who arranged serum collection from the Staff Clinic, Alfred Hospital (Melbourne, Australia); and to international endocrinologists; Drs. N. McClean, S. Salisbury, A. Shlossberg, and R. Rittmaster from Dalhousie University (Halifax, Canada), and Drs. R. Lechan and N. Sauter from Tufts University (Boston, MA). The help of Ms. Kate Dunster in performing the immunofluorescence assays for the (nonpituitary) autoantibodies, of Dr. Jenny Rolland in interpreting the other immunofluorescence assays for the (nonpituitary) autoantibodies, of Dr. Jenny Rolland in interpreting these immunofluorescence assays, of Dr. Damien O’Dwyer for assaying the rheumatoid arthritis sera, and of Ms. Lynn Francis for help with statistical analysis is gratefully acknowledged. Many thanks to Dr. Ross McE. Anderson for reviewing the histopathology of the biopsy-proven cases of hypophysitis, and to Prof. James W. Coding for helpful advice in the earlier stages of this work.

References