Lymphocytic adenohypophysitis presenting as infertility

Case report

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The authors report a nulliparous patient presenting with infertility and hyperprolactinemia. She underwent transphenoidal surgery after radiological investigation disclosed an enlarged pituitary gland which did not respond to bromocriptine therapy. The removed tissue had histological features consistent with adenohypophysitis including a diffuse lymphocytic infiltrate. The lymphocyte subsets present in the infiltrate were characterized by immunohistochemical methods to establish the contribution of different elements of the cellular immune response.

Lymphocytes bearing CD4 antigen (helper-inducer cells) were most prominent and appeared to bear the majority of the interleukin-2 receptor (expressed during lymphocytic activation) present in the pituitary gland. A few B lymphocytes were also observed. The location of the major histocompatibility antigen (classes I and II) and interleukin-2 receptor correlated with the lymphocytes and macrophages rather than with the stromal or parenchymal elements of the pituitary.

Lymphocytic adenohypophysitis is an unusual cause of pituitary enlargement which can mimic a pituitary tumor, and is sometimes associated with hyperprolactinemia. In women of child-bearing age, it almost always occurs during pregnancy or the postpartum stage. The autoimmune disorder reported here has not previously been associated with infertility nor has the lymphocytic infiltrate of the pituitary previously been analyzed in detail by modern immunological methods.

KEY WORDS • lymphocytic adenohypophysitis • adenohypophysitis • infertility • pituitary gland • transsphenoidal surgery • lymphocyte

LYMPHOCYTIC adenohypophysitis is an autoimmune disorder in which the cellular and humoral arms of the immune response attack the anterior lobe of the pituitary gland. Over 30 cases have been confirmed by biopsy or autopsy since the first report of an incidental postmortem finding in 1962.\textsuperscript{7,10,26} All but one of the patients have been women.\textsuperscript{12} Most of the patients presented during pregnancy or the postpartum period with hypopituitarism and enlargement of the pituitary seen on radiological studies; hyperprolactinemia has been found in a number of cases.\textsuperscript{1,2,16,26,27,30,31,34} Thus, the disease often mimics a nonsecreting or prolactin-secreting pituitary adenoma. The patient reported here is the first to present with infertility associated with hyperprolactinemia and an intrasellar mass which, at operation, was shown to be the pituitary gland affected by adenohypophysitis.

In prior reports, pathological examination of affected glands has revealed interstitial fibrosis, destruction of the cells of the anterior lobe, and a diffuse infiltrate of lymphocytes with scattered dense collections resembling lymphoid follicles.\textsuperscript{1,2,7,27} An immunological analysis of the lymphocyte subtypes involved in this infiltrate may be suggestive of ways to diminish or abolish the autoimmune events that result in pituitary dysfunction; however, it has been performed only once before.\textsuperscript{16}

In order to quantify the contributions of the various cellular components of the immune response to this phenomenon, we examined the tissue from our patient by immunohistochemical methods.

Case Report

This 29-year-old nulliparous woman tried unsuccessfully to conceive for 14 months before submitting to a workup for infertility. A uterine fibroid had been removed 6 months earlier.

Examination

Hysterosalpingography revealed patent fallopian tubes and a normal uterus and adnexae. The patient's menstrual cycles had been regular since menarche. She complained of mild galactorrhea and admitted to suf-
fearing bifrontal headaches. Her serum prolactin level was 93 ng/ml (normal < 25 ng/ml). Other tests of endocrine function, which included levels of thyroid hormones, cortisol, and growth hormone, were normal on baseline and provocative testing, as were basal levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Visual fields were intact on formal perimetry, and general physical and neurological examinations were normal. Computerized tomography and magnetic resonance (MR) imaging revealed pituitary enlargement (11 mm in maximum vertical span) with an upward convexity of the superior surface of the gland (Fig. 1). Bromocriptine therapy for 3 months caused a fall in serum prolactin to normal levels, but no change in the size of the pituitary gland as assessed by repeat MR imaging.

Operation and Postoperative Course. Transsphenoidal exploration of the sella revealed an 8-mm well-defined yellowish mass in the anterior lobe of the gland which was removed. Upon pathological examination of this tissue a diagnosis of lymphocytic adenohypophysitis was established. Serum prolactin levels were normal 3 months later and the patient conceived successfully without further medical therapy, delivering a healthy, full-term baby boy 11 months after surgery. At her most recent follow-up examination 15 months after operation, the serum prolactin level was normal and MR imaging showed a pituitary gland of normal appearance.

Endocrine and Immunological Histochemical Studies. Part of the biopsy specimen was fixed in 10% neutral buffered formalin, embedded in paraffin, and cut into sections 8 μ thick. With standard techniques, the sections were stained with hematoxylin and cosin, Laidlaw's connective tissue stain (for reticulin), and antisera against prolactin, corticotropin, and growth hormone. Normal pituitary gland was also stained with these antisera for a control study.

The remainder of the tissue removed at surgery was snap-frozen in isopentane chilled to −70°C. Sections 15 μ thick were cut on a cryostat, exposed to gentle heat for 5 minutes, then fixed in acetone at −20°C for 10 minutes. All subsequent steps were carried out at room temperature. Endogenous peroxidase was quenched by immersion for 5 minutes in 3% hydrogen peroxide in methanol. The general avidin-biotin peroxidase complex (ABC) technique of Hsu, et al., was then followed with some modifications. After washes in 50 mM Tris buffer (pH 7.6), the sections were placed in 2% goat serum in Tris for 5 minutes to block nonspecific binding. Primary mouse-antihuman monoclonal antibodies (Table 1) were applied to the sections for 2 hours in a humid chamber; the Tris and goat serum washes were then repeated. The specificities of the primary antibodies were confirmed by fluorescent cell sorting against resting and stimulated human lymphocytes before use in histochemistry. Goat-antimouse immunoglobulin G F(ab')₂-biotin* was then applied as a secondary antibody for 30 minutes. Reagents from a standard Vectastain ABC kit† were used to label the antigen-antibody complexes with peroxidase, and 3,3'-diaminobenzidine was applied causing deposition of an insoluble brown product at the sites of labeled antigen in each section. Light counterstaining with Mayer's hematoxylin was then performed. Negative control sections were produced by replacing primary antibody with mouse serum (1:200) or antibodies against the same markers in an irrelevant series (rat). Positive control sections were produced on rhesus monkey spleen as a tissue substrate. The density of labeled profiles was established for each antibody by direct counting with an ocular grid in each of six randomly chosen areas in six serial tissue sections.

The parenchymal elements of the anterior lobe were found to be generally atrophic and difficult to identify; moderate edema and fibrosis were present (Fig. 2). Little staining for prolactin, corticotropin, or growth hormone

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**TABLE 1**

Primary antibodies used in immunohistochemistry

<table>
<thead>
<tr>
<th>Target</th>
<th>Antigen Cluster</th>
<th>Clone</th>
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<tbody>
<tr>
<td>class I MHC</td>
<td>none*</td>
<td>W6/32</td>
</tr>
<tr>
<td>class II MHC</td>
<td>none*</td>
<td>4A12</td>
</tr>
<tr>
<td>macrophage/monocyte</td>
<td>CD14</td>
<td></td>
</tr>
<tr>
<td>B lymphocyte</td>
<td>CD19</td>
<td>HD37</td>
</tr>
<tr>
<td>T lymphocyte (pan T)</td>
<td>CD5</td>
<td>T101</td>
</tr>
<tr>
<td>helper-inducer T cell</td>
<td>CD4</td>
<td>OKT4</td>
</tr>
<tr>
<td>cytotoxic-suppressor T cell</td>
<td>CD8</td>
<td>OKT8</td>
</tr>
<tr>
<td>interleukin-2 receptor</td>
<td>CD25</td>
<td>2R1.2</td>
</tr>
</tbody>
</table>

* No cluster of differentiation has been assigned to major histocompatibility (MHC) antigens.
† Clone not specified by manufacturer.
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![Image of photomicrograph](image1)

**Fig. 2.** Photomicrograph of the surgical specimen demonstrating edema, fibrosis, and diffusely distributed lymphocytic infiltrate on a background of atrophic pituicytes; the hallmarks of lymphocytic adenohypophysitis. H & E. × 90.

was present, and no adenomatous tissue was found. A heavy lymphocytic infiltrate found in the biopsy specimen was diffusely distributed; a few areas of loose coalescence suggested follicle formation. An occasional plasma cell was seen. No epithelioid or multinucleated giant cells were found.

The mean densities of lymphocytes, macrophages, and the main lymphocyte subsets (helper-inducer, cytotoxic-suppressor, and B cells) are given in Table 2. Helper-inducer CD4-positive lymphocytes formed the majority of infiltrating cells (Fig. 3 left) and their number exceeded that of cytotoxic-suppressor CD8-positive cells by almost 2:1. Macrophages comprised a lesser component of the infiltrate and only an occasional B cell was seen. Most of the class II major histocompatibility (MHC) antigen found in each section correlated geographically with lymphocyte and macrophage antigens (Fig. 4). Class I MHC antigen was present on both types of infiltrating cell and also to a much lesser extent on the parenchymal and/or stromal elements of the gland itself. Interleukin-2 (IL-2) receptor was scattered throughout the sections in a pattern similar to that shown by the T lymphocytes and, in particular, the CD4-positive subset (Fig. 3 right). Occasional focal areas of accentuation matched the locations of lymphocyte concentrations shown by routine histochemistry. The distribution of IL-2 receptor and class II MHC antigen in each section followed the distribution of lymphocytes and macrophages but could not be associated with either cell type alone.

### Discussion

**Infertility and Adenohypophysitis**

Lymphocytic adenohypophysitis is an uncommon autoimmune disorder, the natural history of which is unknown. Its discovery mainly during pregnancy, in the postpartum period, or as an incidental finding at autopsy confers distinction on the patient reported here.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Density</th>
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<tbody>
<tr>
<td>T lymphocytes</td>
<td>1520 ± 276</td>
</tr>
<tr>
<td>CD2+</td>
<td>924 ± 200</td>
</tr>
<tr>
<td>CD4+</td>
<td>540 ± 78</td>
</tr>
<tr>
<td>CD8+</td>
<td></td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Macrophages</td>
<td>289 ± 83</td>
</tr>
<tr>
<td>Interleukin-2 receptors</td>
<td>448 ± 108</td>
</tr>
<tr>
<td>Major histocompatibility antigens</td>
<td></td>
</tr>
<tr>
<td>Class I</td>
<td>1280 ± 208</td>
</tr>
<tr>
<td>Class II</td>
<td>988 ± 194</td>
</tr>
</tbody>
</table>

* Mean number of discrete antigen profiles, ± standard deviation, per high-power (×150) field in six randomly chosen fields.
Only two other women of child-bearing age who had never been pregnant have been reported with this disease. In those two cases, the patients presented with amenorrhea; they may have been infertile but the authors did not address that issue. One remained amenorrheic after surgery, while the clinical outcome of the other patient was not provided. The patient described here conceived 2 months after excision of an inflammatory mass from her pituitary gland. She showed no stigmata of the several autoimmune diseases (in particular, thyroiditis) with which adenohypophysitis has previously been associated.11,12,14,21-26,32,34

Adenohypophysitis could have caused this patient's amenorrhea and infertility by two possible mechanisms. First, she may have had direct impairment of the pituicytes responsible for secreting LH and FSH. Although serum levels of these hormones were normal, the inflammation may have altered the ovulatory response of the gonadotrophs to steroid signals from the ovarian follicle, or to the pulsatile secretion of hypothalamic gonadotropin-releasing factor (GnRF) which regulates this process. The pituitary gland, therefore, may not have been able to provide the surge of these hormones that implantation requires. The effect of selective hormonal dysfunction on corticotrophs has been observed in other patients with adenohypophysitis.16 and LH/FSH dynamics may have been similarly selectively unpaired in our patient.

Hyperprolactinemia provides a second possible explanation for infertility in this case. Elevated serum levels of prolactin have often been noted with this disease16,26,30,31,36, and may produce galactorrhea.16,30,31 Excess prolactin causes central anovulatory dysfunction, which produces an inadequate luteal phase and anovulation. High levels of prolactin cause cessation of menses by complete suppression of GnRF.35 Many women with prolactin-secreting pituitary tumors achieve pregnancy after tumor removal induces a fall in the serum levels of prolactin.48 A similar mechanism may have helped our patient to conceive after removal of the mass from her pituitary gland.

Hyperprolactinemia in Adenohypophysitis

In the case reported here, inflammation is unlikely to have been provoked by excess prolactin; rather, the high levels of prolactin probably appeared as a consequence of the disease. The inflammatory process may have had a stimulatory effect on lactotrophs. It could also have interfered with prolactin-inhibiting factors at their receptor, or a stimulatory antibody for prolactin release may have been produced by B cells participating in the autoimmune process. Antipituitary antibodies have been detected in women with Sheehan's syndrome18 and in patients with polyglandular autoimmune syndromes who had no clinical evidence for adenohypophysitis.4 Antibodies against the pituitary have also been detected in three patients with lymphocytic adenohypophysitis.23,25,36

Mechanical factors alone could account for the hyperprolactinemia in our patient. The enlarged gland or the inflammatory mass may compress the adjacent stalk and interfere with the delivery of prolactin-inhibiting factors through the portal venous system. Surgical removal of the focus of inflammation may have caused resolution of hyperprolactinemia in our patient by relieving the stalk compression and thereby allowing the physiological regulation of prolactin secretion to resume. It is also possible that removing some of the infiltrating lymphocytes reduced any direct inhibitory effect that they may have had on pituicytes. Whatever the etiology of the pituitary dysfunction, it seems reasonable to biopsy these portions of the gland which, by virtue of their swelling, resemble tumor (as was done in this patient) and thereby enhance the chances of reestablishing normal endocrine function. McGrail, et al.,24 reported one other patient who regained anterior pituitary function after biopsy. They suggested that recovery may have occurred spontaneously. Not enough is known about the natural history of this disease to draw firm conclusions in this regard.

Cellular Infiltrate in Lymphocytic Hypophysitis

Cellular participants in the immune response include macrophages and T lymphocytes. Pluripotential stem cells of the T lineage develop into two predominant classes: the helper-inducer cell identified by the presence of the CD4 protein on its surface, and cytotoxic-suppressor cells that bear the CD8 protein. In general, CD4-positive cells modulate and magnify the cytolytic effector role performed by the CD8-positive cells. Together, they form the cellular arm which clears foreign, nonsoluble antigen from the organism. The B cells, which produce antibodies, probably have only an indirect effect on the cellular immune response.

The immunological characteristics of the cellular infiltrate have been studied in only one other patient.
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with adenohypophysitis. In that patient the CD4-positive:CD8-positive cell ratio was 2:1 and CD4-positive cells were seen only within lymphoid follicles, which also contained B cells. Cells with a histiocytic morphology were observed around degenerating pituicytes in association with a mix of lymphocyte types, but no quantification or staining for macrophage markers or histocompatibility antigens was carried out. In the patient reported here, a similar CD4-positive:CD8-positive cell ratio was found, but CD4-positive cells were seen both within sparse accumulations resembling lymphoid follicles and in the sparser background infiltrate. The few B cells present were scattered at random throughout the specimen, as were macrophages.

This predominance of T cells over B cells and macrophages is in keeping with the characteristics of infiltrates in other autoimmune diseases.\(^1,9,15,21\) The CD4-positive:CD8-positive cell ratio depends upon the organ affected and, within an organ, upon the specific disease process. In Sjögren’s syndrome (and Graves’ disease), CD4-positive cells predominate;\(^15,21\) in pancreatic insulitis (and Hashimoto’s thyroiditis), CD8-positive cells are more numerous.\(^3,9\) Animal models of adenohypophysitis have been produced in several species but in none has the infiltrate been characterized by immunological methods.\(^19,20\)

The IL-2 receptor is a marker of activation in lymphocytes.\(^18\) In allografts undergoing acute rejection, the expression of IL-2 receptor was found to be above basal levels in infiltrating T cells;\(^22,23\) however, it has not previously been studied in organs undergoing autoimmune attack. Analogy with allograft rejection suggests that, if lymphocytes are sensitized to and activated by antigen within the pituitary gland, they should express the IL-2 receptor. Its presence in our patient thus supports an active role for the cellular arm of the immune response in this disease.

MHC Antigens and Pituitary Autoimmunity

The MHC complex comprises a set of immunoregulatory molecules intimately linked with the presentation of antigen to effector lymphocytes and with the distinction between self and nonself. It exists in two forms: on the surface of all cells as class I, and selectively as class II on cells with an actual or potential role in the immune response. Class II differences between an allograft and the cellular immune system of its host lead to recognition of the graft as foreign. The allograft is then presented as such to cytotoxic T lymphocytes by macrophages that identify the appropriate effectors through the congruity between their own class II MHC antigen and that of the effector cell.

Histocompatibility antigens are relevant to autoimmunity in light of the theory advanced by Bottazzo, et al.,\(^2\) that endocrine cells inappropriately expressing class II MHC might provoke such an immune response by presenting their own surface antigen to T cells. Such inappropriate expression of class II MHC has been shown in several organs undergoing autoimmune attack,\(^3,15,21\) but it was not apparent in the present case of lymphocytic adenohypophysitis. Class II MHC expression may be an effect of inflammation rather than a cause. Most class II MHC is found on lymphoid and endothelial cells; it has been reported as absent in normal pituitary tissue.\(^8\) It is possible that pituicytes lack the capacity in vivo to increase their expression of this antigen. If such an observation is confirmed in other patients, the universal application of this theory of autoimmune etiology must be questioned.

To improve the treatment of lymphocytic adenohypophysitis, some method of preoperative diagnosis must be found and a better understanding of the natural history of the disorder attained. One patient improved after surgery, an outcome which may have been coincidental or, more likely, the result of decompression of the pituitary stalk. In the future, it may be possible to aim ablative or inhibitory therapies at specific cellular targets in the immune response, but this should be studied in available animal models. It is clear that this disease can occur in young women without prior pregnancy, and that it may prevent pregnancy through direct derangement of gonadotropin secretion or, secondarily, by causing hyperprolactinemia. Although it is most frequently found in association with pregnancy, the diagnosis of adenohypophysitis should be considered in any woman with an enlarged pituitary gland, especially if evidence for other types of autoimmune disease is present.

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References


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