Adenohypophysitis in rat pituitary allografts

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Summary

The histological, immunohistochemical and ultrastructural alterations in 81 pituitary allografts from Lewis rats transplanted beneath the renal capsule of Wistar rats were investigated. Intrasellar pituitaries of rats bearing allografts were also examined. Recipient rats were sacrificed at various time points after transplantation. Two days after transplantation, the central portion of the allografts demonstrated ischaemic necrosis. A week later, massive mononuclear cell infiltrates consisting primarily of lymphocytes and to a lesser extent, macrophages, plasma cells and granulocytes became prominent. At about three to four weeks after transplantation, the mononuclear cell infiltrate diminished; the surviving adenohypophysial cells, mainly prolactin (PRL) cells, increased in number and necrosis was replaced by connective tissue. No histological changes were noted in the intrasellar pituitaries of rats bearing allografts. Immunohistochemistry demonstrated that the surviving adenohypophysial cells were mainly PRL-producing cells. Electron microscopy revealed adenohypophysial cell destruction, a spectrum of inflammatory cells and, in late phase, accumulation of fibroblasts and collagen fibres. PRL cells were the prominent cell type; they increased in number. It appears that pituitary allografts are ‘foreign’ and evoke an immune response, suggesting that they may be used as an experimental animal model for morphological investigation of the development and progression of adenohypophysitis, a rare disease occurring mainly in young women often associated with pregnancy.

Keywords
allograft, autoimmunity, inflammation, pathology, pituitary

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Inflammatory and infectious lesions affecting the pituitary are varied in nature, but rare (Sautner et al. 1995; Thoudou et al. 1995; Cheung et al. 2001; Rivera 2006; Molitch & Gillam 2007). Bacteria, fungi and even protozoa are recog-
and in association with regional neoplasms, particularly craniohypophysial tumors (Sonnet et al. 2006; Nishikawa et al. 2007). Pituitary adenomas only infrequently engender an inflammatory response.

The most intriguing inflammatory lesion affecting the pituitary is chronic lymphocytic hypophysitis (Asa et al. 1981; Sautner et al. 1995; Thoudou et al. 1995; Catusergi 1998; Cheung et al. 2001; Catusergi et al. 2005; Rivera 2006; Molitch & Gillam 2007). It affects most frequently young women and is often associated with pregnancy. Clinical effects include pituitary enlargement, headache and visual disturbance. If untreated, the process leads to various degrees of hypopituitarism. In early reports, a lethal outcome was occasionally reported and attributed to loss of adrenocorticotropic hormone (ACTH) secretion and adrenocortical failure (Goudie & Pinkerton 1962). In some cases, chronic inflammation extends to involve the posterior lobe and pituitary stalk, a process termed 'infundibuloneurohypophysitis' accompanied by diabetes insipidus and characterized by thickening of the pituitary stalk on neuroimaging (Imura et al. 1993). Although chronic lymphocytic hypophysitis is regarded as an autoimmune disease, a number of issues remain unresolved (Thoudou et al. 1995; Catusergi 1998; De Bellis et al. 2005a; Molitch & Gillam 2007). These centres upon aetiology, immunological and morphological evolution, as well as establishment of preoperative diagnostic criteria and more effective therapies.

The morphological features of lymphocytic hypophysitis and its association with other endocrine autoimmune diseases suggested that it represents an autoimmune disorder. It is a component of the autoimmune polyglandular syndrome, known to be due to mutational inactivation of the Autoimmune Regulator (AIRE) gene (Mathis & Benoist 2007). However, the target antigen remains to be identified.

The aim of our study was to induce pituitary inflammation in rats and to investigate the evolution and progres-

sion of the resulting lesions in terms of histology, immunohistochemistry and electron microscopy. To achieve our goal, we transplanted adenohypophysial tissue of Lewis rats beneath the renal capsule of Wistar rats. We hypothesized that an inflammatory reaction similar to that of lymphocytic hypophysitis would develop in the pituitary allografts.

**Materials and methods**

Pituitaries of female Lewis rats (donors) of 200 grams body weight were implanted beneath the renal capsule of male Wistar rats (hosts) also of 200 grams body weight. Nine groups of nine animals were used to assess the graft response at 2 days, 4 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks and 8 weeks posttransplantation. In addition, five sellar pituitaries of normal Wistar rats and the sellar pituitaries of rats bearing allografts were also investigated.

Prior to initiating the surgical removal of the pituitaries of Lewis rats (donors), the Wistar rats (hosts) were anaesthetized with ether vapours, dorsally shaved and placed ventrally upon the operative surgical table. The right kidney was exposed via a dorsal approach, a small incision was made in the renal capsule and a subcapsular pouch was prepared. The donor Lewis animals were then anaesthetized using sodium pentobarbital and decapitated. The intact pituitary glands were quickly removed. Thereafter, the neurointermediate lobe was discarded and the anterior pituitary lobe, bisected, was placed in isotonic saline solution and promptly allografted deep into the subcapsular pouch of the graft hosts. The kidney was then returned to its place and the dorsal wound was closed. The time between donor decapitation and placement of the pituitary graft beneath the renal capsule did not exceed 60 s. Full recovery of the animals occurred within 20–30 min.

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**Table 1 Overview of Antibodies and Dilutions used in this study**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Manufacturer</th>
<th>Source</th>
<th>Cat. #</th>
<th>Antigen retrieval</th>
<th>Dilution</th>
<th>Incubation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD-3</td>
<td>Dako</td>
<td>Rabbit</td>
<td>A0452</td>
<td>1% Pepsin</td>
<td>1/100</td>
<td>1 h</td>
</tr>
<tr>
<td>CD-45</td>
<td>BD Pharmingen</td>
<td>Mouse</td>
<td>550567</td>
<td>H.I.E.R Tris-EDTA pH 9.0</td>
<td>1/300</td>
<td>over night</td>
</tr>
<tr>
<td>CD-68</td>
<td>Serotec</td>
<td>Mouse</td>
<td>MCA341R</td>
<td>1% Pepsin</td>
<td>1/600</td>
<td>1 h</td>
</tr>
<tr>
<td>F-8</td>
<td>Dako</td>
<td>Rabbit</td>
<td>A0082</td>
<td>H.I.E.R Tris-EDTA pH 9.0</td>
<td>1/200</td>
<td>1 h</td>
</tr>
<tr>
<td>mGH</td>
<td>National Hormone &amp; Peptide Program</td>
<td>Rat</td>
<td>AFP 5672099</td>
<td>No pretreatment</td>
<td>1/100</td>
<td>1 h</td>
</tr>
<tr>
<td>mPRL</td>
<td>National Hormone &amp; Peptide Program</td>
<td>N.A.</td>
<td>No pretreatment</td>
<td>1/300</td>
<td>1 h</td>
<td></td>
</tr>
<tr>
<td>ACTH</td>
<td>Abcam</td>
<td>Rabbit</td>
<td>Ab5407</td>
<td>H.I.E.R Tris-EDTA pH 9.0</td>
<td>1/400</td>
<td>1 h</td>
</tr>
</tbody>
</table>


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After surgery, the host animals were injected intramuscularly with penicillin (Procaine penicillin; 5,000 IU intramuscularly, Lakeside Pharma Inc., Edo Mexico, Mexico) daily for 3 days. Following the course of antibiotic treatment, the grafted animals were deeply anaesthetized with sodium pentobarbitral and were decapitated at 2 and 4 days as well as at 1, 2, 3, 4, 5, 6 and 8 weeks postgraft placement. The sellar pituitary and the portion of the kidney containing the pituitary graft were rapidly removed, fixed in 10% neutral buffered formalin and were routinely processed before being paraffin-embedded.

Sections of 4–6 μm thickness were cut and stained with haematoxylin-eosin (H&E). All slides were immunostained using the streptavidin-biotin peroxidase complex method and the antibodies with respective sources, dilutions and ancillary methods described in Table 1. Details of the methods used for immunohistochemistry and electron microscopy were described in previous publications (Rotondo et al. 2006, 2008).

Results

Histological examination of 2 day grafts showed extensive central necrosis (Figure 1). The cell borders of secretory cells were not recognizable, but nuclear remnants remained visible. At 4 days, necrosis advanced, nuclear remnants were no longer apparent. No extensive inflammatory infiltration was seen. At 1 week and 2 weeks, massive inflammatory infiltration mainly composed of lymphocytes and to a lesser extent, macrophages, plasma cells and granulocytes was evident Figure 2. Immunohistochemistry showed that a majority of inflammatory cells were CD3-immunopositive T-lymphocytes Figure 3. Several B-lymphocytes immunopositive for CD-45 and macrophages reactive for CD-68 were also noted Figure 4. At later phases, the size of the necrotic zone gradually diminished and became replaced by fibroblasts and collagen fibres Figure 5. The number of inflammatory cells was also decreased. The surviving adenohypophysial cells, however, were more numerous and the peripheral rim of

Figure 1 Two days after transplanting a pituitary under the kidney capsule, acute ischaemic necrosis and several dilated vessels are seen in the graft. Haematoxylin-eosin (H&E); Magnification: 200x.

Figure 2 Two weeks after transplantation, inflammatory infiltration predominates. No inflammatory cells are noted in the renal parenchyma. Haematoxylin-eosin (H&E); Magnification: 200x.

Figure 3 Immunostaining demonstrates that many inflammatory cells are CD-3 immunopositive T-lymphocytes. Two weeks after transplantation; immunostaining for CD-3; Magnification: 200x.
Figure 4 Immunostaining shows CD-68 immunopositive macrophages. Two weeks after transplantation; immunostaining for CD-68; Magnification: 400x.

Figure 6 Six weeks after transplantation, the surviving portion of the graft increased in size and shows the dominance of prolactin (PRL) immunopositive cells. Immunostaining for PRL; Magnification: 100x.

Figure 5 Six weeks after transplantation, the necrotic area regressed in size and is replaced by connective tissue. The inflammatory infiltration is not conspicuous. Haematoxylin-eosin (H&E); Magnification: 200x.

Figure 7 Allograft showing marked inflammatory response (upper right and lower right corners). Note the markedly enlarged hyperplastic (asterisk), prolactin producing (PRL) cell. Magnification: 4000x.

Parenchyma appeared to increase in depth. Immunostaining for hormones showed many of the secretory cells to be immunoreactive for PRL Figure 6. The immunopositive material was localized mainly in the paranuclear Golgi zone. Such 'Golgi pattern' staining is a characteristic feature of actively secreting PRL cells. Immunostaining also demonstrated a few immunopositive ACTH and fewer growth hormone (GH) cells.

Electron microscopy (EM) documented adenohypophysial cell destruction in early phases of the process followed by the accumulation of inflammatory cells. In later phases, fibroblasts and collagen fibres became prominent features. Most of the secretory cells were PRL cells, although a few examples of other adenohypophysial cell types were also
recognized. At late phase, PRL cells appeared to increase in number. Ultrastructurally, they showed evidence of endocrine activity Figures 7 and 8.

The sellar pituitaries of rats bearing an allograft showed no abnormality and there was no detectable difference between those glands and the glands of normal control rats.

Discussion

A number of experimental studies have documented the induction of inflammatory lesions of the pituitary in various animal models. Levine injected pituitary extracts in Freund adjuvant in rats and thereafter demonstrated extensive inflammation in the anterior lobe of the pituitary (Levine 1967, 1969). Levine termed the lesion “allergic adenohypophysitis” and suggested an autoimmune aetiology. Subsequently, Onodera et al. induced pituitary inflammation in mice infected with Reovirus type 1 (Onodera et al. 1981). The animals developed not only growth retardation but also diabetes and gastritis. Their pituitaries featured coagulative necrosis and mononuclear cell infiltrates. Furthermore, viral particles were demonstrated within GH-producing cells. The experimental induction of pituitary inflammation using various methods were also reported by others (Lee 1977; Klein et al. 1982; Yoon et al. 1992; Watanabe et al. 2001; Davis et al. 2002; de Jersey et al. 2002). The morphological findings in autoimmune hypophysitis resemble those of graft rejections. Both conditions result from immunological injury aiming to eliminate tissues which become foreign to the organism. More recent studies have identified a number of putative antigens that might be responsible for the development of an autoimmune response, including alpha- and gamma-enolase (O'Dwyer et al. 2002), neuron specific enolase (Bensing et al. 2005), tudor domain containing protein 6 (TDRD6) (Bensing et al. 2007).

Tzou et al. (2008) published the results of a detailed study inducing autoimmune hypophysitis in mice. These authors immunized female SJL mice with mouse pituitary extracts emulsified in Freund adjuvant and succeeded in inducing massive mononuclear cell infiltration and adenohypophysial cell death. The process was associated with pituitary enlargement and hypopituitarism. The intermediate and posterior lobes were uninolved by inflammation. The inflammatory cells consisted of CD3-positive T-lymphocytes and CD-45 positive B-lymphocytes. Granulocytes and plasma cells were also noted. In circulating blood of these mice, several proteins were identified that may have functioned as pituitary auto-antigens.

Our results clearly show the development of massive inflammatory reaction in pituitary allografts transplanted from Lewis rats to Wistar rats. The findings are not unexpected in that pituitary tissues of Lewis rats are immunologically ‘foreign’ to Wistar rats. At 1–2 weeks, the process closely resembled that seen in human autoimmune adenohypophysitis (Asa et al. 1981; Saunier et al. 1995; Thoudou et al. 1995; Cheung et al. 2001). Thus, it may represent a possible animal model of the condition. There are, however, differences in early and late phases of the experimental process. Massive ischaemic necrosis is seen at the centre of the grafts within days of transplantation, not a surprising finding as the grafts are obviously not well vascularized. In the human disease, the pituitary is studied histologically only in the later phases and it is not known whether a phase of necrosis precedes the inflammatory infiltration. The late-phase difference is that in human lymphocytic hypophysitis, the lesion is progressive, inflammatory infiltration persists and the loss of adenohypophysial cells are replaced by massive accumulation of connective tissue. In contrast, the rat model features a decrease in inflammation, and the surviving PRL-producing cells increase in number. Obviously, more work is needed to determine whether the nature of the autoimmune lesion is similar in the animal model to the cellular and humoral process active in human lymphocytic hypophysitis.

Aside from the inflammation, an intriguing finding in our animal model was the marked increase in the number of
PRL-producing cells around the necrotic focus. Increase in PRL cell number in pituitary transplants has previously been reported (Kovacs 1961; Lombardero et al. 2006, 2009; Tzou et al. 2008). It was reasonably suggested that PRL cell hyperplasia is due to the loss of the inhibitory effect of the down-flow of hypothalamic dopamine, the consequence of interrupting hypothalamic and pituitary continuity.

Recent evidence indicates that PRL possesses a considerable effect upon immune mechanisms (Berczi 1993, 1994; De Bellis et al. 2005b). It is known that PRL stimulates angiogenesis by decreasing the expression of thrombospondin, an angiogenesis inhibitor (Johansson et al. 2009). PRL plays an important role in the revascularization, increasing blood vessel density, blood perfusion and oxygen tension. It also facilitates engrafting of pancreatic islet cells in that PRL is mitogenic in such cells (Johansson et al. 2009). PRL suppresses pancreatic islet cell apoptosis, enhances B-cell proliferation and is involved in the induction of islet B cell hyperplasia (Johansson et al. 2009). Furthermore, it is responsible for the maintenance of normal glucose homeostasis during pregnancy by causing an increase in B-cell mass (Huang et al. 2009). Whether PRL can affect other cell types in the adenohypophysis remains to be investigated.

Prolactin is involved in endothelial cell proliferation, migration, protease production and apoptosis (Davis et al. 2002; Rivera et al. 2008). It can disrupt the actin cytoskeleton, induce changes in cell shape, reduce cell adhesion, transendothelial granulocyte trafficking and act on endothelial D2 receptors. Endothelial cells can express PRL mRNA and release PRL (Clapp et al. 1998). PRL receptor is expressed in vascular endothelium and PRL affects immune responses to several antigens (Corabacho et al. 2002; Rivera et al. 2008). Obviously, more studies are required to reveal the role of PRL in the progression of adenohypophysitis.

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