EVIDENCE FOR CELLULAR MEDIATED IMMUNITY IN AN ANIMAL MODEL OF AUTOIMMUNE PITUITARY DISEASE

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Abstract

The anterior pituitary gland can be involved in an inflammatory reaction mediated by lymphocytes that leads to various degrees of dysfunction. In seven rabbits immunized with homologous pituitary tissue in complete Freund's adjuvant, a focal lymphocytic infiltrate, and increased fibrosis was observed in five. These changes were patchy in distribution and limited to the anterior pituitary. No inflammation was observed in five control animals. When incubated with pituitary extract, partially purified lymphocytes from four of the five animals with altered pituitary histology demonstrated a significant (p < 0.05) stimulation of \( ^{3}H \)-thymidine incorporation. Measures of antipituitary antibodies using indirect immunofluorescence were negative in experimental as well as control animals. The present studies characterize the histologic changes and suggest that cellular immunity plays a role in the pathogenesis of experimentally induced autoimmune pituitary disease.

Introduction

There are various pathologic processes that can effect the pituitary gland and alter its function (1, 2). Recent reports suggest that similar to other endocrine glands, there is a chronic autoimmune disease that involves the anterior pituitary (3-5). This process is characterized by variable degrees of pituitary dysfunction, occasionally enlargement of the sella turcica, and histologically by the presence of a lymphocytic cellular infiltrate and increased fibrosis giving rise to the term lymphoid...
Anterior hypophysitis (3-7). This disease may occur more commonly in human population than previously thought. In order to better understand the possible immunologic mediators of this disease, we have produced an animal model in rabbits immunized with homologous pituitary tissue. The present report characterizes the histologic changes in this animal model and gives evidence to support a cellular mediated immune mechanism for this disease.

Methods

Female New Zealand white rabbits were immunized with homologous rabbit pituitary tissue. The pituitary tissue for immunization was obtained immediately after sacrifice of three control animals, Dounce homogenized in phosphate buffered saline and stored at -80°C until use. At the time of immunization the whole pituitary homogenate was mixed with an equal volume of complete Freund’s adjuvant to yield a final protein concentration of 2.0 mg/ml. Each experimental animal received 1 ml administered intradermally to multiple sites on the back. Control animals received either Freund’s adjuvant diluted 1:1 with PBS (C1, C2, and C3) or homologous liver tissue (C4 and C5) in complete Freund’s adjuvant (protein conc = 2.0 mg/ml). Immunizations were repeated at two-week intervals for a total of three immunizations each time utilizing complete Freund’s adjuvant, a total volume of adjuvant plus antigen of one ml. The second and third injections were given at a combination of subcutaneous and intramuscular sites on the animal’s back.

Prior to immunization, 20 ml of blood was removed under sterile conditions from the ear vein of each animal into a syringe containing 1,000 units of heparin. An additional 10 ml of blood was obtained without anticoagulation, and after clotting the serum was collected by
centrifugation at 1,000 xg for 20 minutes and frozen at -20°C. Then either 8 weeks (10 animals: 6 experimental, EI-E6 and 4 controls, C1-C4) or 16 weeks (2 animals) after the initial immunization, repeat blood samples were obtained and the animals sacrificed by decapitation. The pituitaries were removed intact and fixed in 10% formalin, embedded in paraffin and then stained with hematoxylin and eosin, Masson trichrome or periodic acid-Schiff/hematoxylin-orange G (orange G-PASH) (9). The glands were examined in both the control and experimental animals to evaluate presence of lymphocytic infiltration and for the presence of fibrosis and for any other histological abnormalities.

Cellular immunity was assessed using lymphocytes purified from heparinized blood obtained both prior to immunization as well as prior to sacrifice. Lymphocytes were isolated by centrifugation of 10 ml of blood layered over 3 ml of ficoll-hypaque (density 1.077) at 1,000 xg for 30 minutes in a Beckman Model TJ-6 Centrifuge (10). The cells were suspended in 20% fetal calf serum containing media to yield a final cell count of 1 x 10^6 cells/ml. An aliquot of media containing 2 x 10^5 cells is added to each 1 x 0.6 cm well in a plastic multwell tissue culture plate and subsequently 10 µl of the antigen to be assayed. These antigens include phytohemagglutinin (PHA) 0.9 mg/ml, whole pituitary extract (1.8 mg/ml) and albumin (2.0 mg/ml). Lymphocyte stimulation was measured as described by Cormu, et al utilizing ^3H-thymidine incorporation by cells in culture (10). Basal activity was measured in the absence of added antigens. Each assay was performed in quadruplicate and the results expressed as a function of the unstimulated basal ^3H-thymidine incorporation.
(stimulation index). Statistical significance was assessed by the
student "t" test for paired samples, and 3H-thymidine incorporation
was considered significantly stimulated when p < 0.05.

Humoral immunity was studied by assaying individual animal sera
for the presence of antipituitary antibodies. Serial dilutions of
preimmune sera as well as sera obtained prior to sacrifice were
incubated with sections of normal rabbit anterior pituitary. The
individual pituitary sections were washed with PBS and then in-
cubated with a fluorescence conjugated goat antirabbit IgG. After
30 minutes the samples were thoroughly washed and viewed under a
fluorescent microscope for the presence of antibody localization.
In all studies the preimmune sera served as a control.

Serum from both control and experimental animals was assayed
for concentrations of 1-thyroxine and cortisol by radioimmunoassay.
Values measured prior to immunization were compared with those
obtained at the time of sacrifice. In addition, values were com-
pared between control and experimental animals. Weights of the
animals were measured every 14 days.

-Results

At the time of sacrifice pituitary glands from five control
and seven experimental animals were studied using hematoxylin and
eosin, orange G-PASH and Masson trichrome staining. None of the
five controls showed histologic abnormalities. Of the seven ex-
perimental animals immunized with homologous pituitary tissue as
described, five had evidence of pituitary inflammation. The
histopathological changes consisted of focal lymphocytic infiltrate
with some plasma cells, few eosinophils and areas of fibrosis.
This cellular infiltrate was occasionally dense (Figure 1). These
Figure 1
Hematoxylin and eosin stained sections of anterior pituitary from a control (A) and an experimental animal (B) immunized with homologous pituitary tissue (Mag x 220).

Changes were limited to the adenohypophysis and never involved the neurohypophysis. The areas of fibrosis did not necessarily coincide with the areas of cellular infiltration. The affected glands were friable and easily disrupted when removal was attempted. Special stains for bacteria, fungi and acid-fast organisms were negative. No electron microscopic or immunoperoxidase studies were performed.

Studies of cellular immunity were performed using rabbit lymphocytes cultured in the absence (basal activity) or presence
of pituitary extract (18 μg protein/incubation well) as described above (Table II). The results are expressed as an index of stimulation comparing 3H-thymidine uptake in PHA and pituitary stimulated cells with that measured in unstimulated cultures. Four experimental animals (1, 3, 4, 7) showed significant stimulation of 3H-thymidine incorporation (p < 0.05) when compared to basal activity. There was no stimulation by pituitary extract of lymphocytes purified from either control or experimental animals prior to immunization. All four animals with an increased stimulation index to pituitary extract had histologic evidence of pituitary inflammation.

**TABLE I**

**EFFECT OF PHA AND PITUITARY EXTRACT ON 3H-THYMIDINE INCORPORATION BY RABBIT LYMPHOCYTES**

<table>
<thead>
<tr>
<th>Animals</th>
<th>PHA</th>
<th>Pituitary Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.60</td>
<td>0.37</td>
</tr>
<tr>
<td>2</td>
<td>2.22</td>
<td>0.60</td>
</tr>
<tr>
<td>3</td>
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<td>0.82</td>
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<td>4</td>
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<td></td>
</tr>
<tr>
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<td>2.60</td>
<td>1.50+</td>
</tr>
<tr>
<td>2*</td>
<td>2.84</td>
<td>1.14</td>
</tr>
<tr>
<td>3*</td>
<td>6.25</td>
<td>1.50+</td>
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<td>0.78</td>
</tr>
<tr>
<td>7*</td>
<td>9.65</td>
<td>2.14+</td>
</tr>
</tbody>
</table>

*Animals with histologic evidence of pituitary inflammation.

*Significantly increased compared to basal activity (p < 0.05).
Antipituitary antibodies were assayed in six of the experimental animals by direct immunofluorescence employing normal rabbit pituitary tissue and using preimmune sera as a control. None of the immunized animals demonstrated any significant degree of immunofluorescent staining at dilutions of sera of 1:2 or greater. Similarly in two experimental animals there was a failure to detect significant amounts of rabbit immunoglobulins in frozen sections of their pituitary tissue utilizing direct immunofluorescence with conjugated goat antirabbit IgG.

Measurements of serum levels of T₄ and cortisol both prior to immunization and at the time of sacrifice showed no significant changes in either control or experimental animals.

**Discussion**

Autoimmunity has been implicated as a pathologic process to explain dysfunction of various endocrine glands (1-4, 11). The present study demonstrates that immunization of rabbits with homologous pituitary tissue can produce an inflammatory infiltrate in the anterior pituitary of experimental but not of control animals. Levine has previously shown that rats vaccinated with pituitary tissue developed a lymphocytic infiltrate localized to the anterior pituitary (12). In those studies no attempt was made to determine which immune components either cellular or humoral played a role in the development of the histologic changes. Our studies extend prior work by showing that lymphocytes isolated from four of seven experimental animals have a sensitivity to homologous pituitary tissue as measured by ³H-thymidine incorporation in cultured cells. This latter technique is used as a measure of lymphocyte blast transformation (10). Under the con-
ditions employed for these experiments PHA provided a qualitatively uniform but quantitatively variable increase in the $^{3}H$-thymidine stimulation index. This degree of stimulation was not significantly different between control and experimental animals. This variability could in part be accounted for by the variation in basal levels of $^{3}H$-thymidine incorporation, which in turn may reflect the effect of immunization with Freund's adjuvant per se, or alternatively the presence of fetal calf serum in the cell cultures (13). The changes in basal activity, however, did not alter the ability to detect significant lymphocyte stimulation in four of the experimental animals. It has been reported that peripheral blood lymphocytes in rabbits show a variable response to sensitizing antigens and this makes the current observation of even greater significance (13). There appears to be fairly good correlation between the production of pituitary histologic changes and the increased stimulation index observed for cultured lymphocytes. In contrast using the technique of indirect immunofluorescence we are unable to show any significant titers of anti-pituitary antibodies in the sera of the pituitary immunized animals. The current report supports a role for cellular mediated immunity in the production of the inflammatory reaction of lymphoid hypophysitis.

In addition to cellular infiltration, we observed increased amounts of fibrous tissue in some of the experimental animals. Despite the presence of inflammation of the pituitary gland, the experimental animals maintained normal serum levels of thyroid hormones and cortisol implying grossly normal pituitary gland function. This observation is not surprising, in view of the focal, patchy nature of the anterior pituitary inflammation.
Perhaps functional hypopituitarism represents only one extreme of the possible results of pituitary autoimmunity. It is interesting to speculate that the combination of relatively normal pituitary function and glandular fibrosis is analogous to that seen in some patients with the empty sella syndrome.

Acknowledgements

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References