Pineal glands of rats with experimentally induced autoimmune uveitis (EAU) were studied histologically. Inflammatory changes, characterized by mononuclear infiltration, were found in the pineal glands of one-third of the Lewis rats that developed EAU by active immunization with S-antigen. No changes in the pineal gland were observed in AVN rats which are "low responders" for EAU and did not develop ocular disease. Frequency and severity of both pineal gland and ocular involvement clearly were elevated by intravenous injection of Bordetella pertussis along with the S-antigen immunization; all B. pertussis-treated rats of both Lewis and AVN strains developed pineal and ocular changes. Inflammatory changes of the pineal gland also were found in rats in which EAU was induced passively by transfer of lymphocytes from S-antigen-immunized donors. The frequency of involvement of the pineal gland was found to be lower than that of the retinas in rats where EAU was induced by active immunization or by adoptive transfer of lymphocytes. Invest Ophthalmol Vis Sci 24:1333-1338, 1983

No data have been reported, to the best of our knowledge, concerning the involvement of the pineal gland in species other than the guinea pig in which experimental autoimmune uveitis (EAU) can be induced. We report here our findings with regard to the histologic changes in the pineal gland of rats immunized with S-antigen. The rats used were of two genetically different strains, with different susceptibility to EAU.9 In addition, we examined the changes in pineal glands of rats in which EAU was induced by transferred lymphocytes from donors immunized with S-antigen.

Materials and Methods

Animals

Lewis rats were purchased from M.A. Bioproducts, Walkersville, MD. AVN rats were offspring of breeding pairs, provided by Dr. David Gasser (University of Pennsylvania). Male and female rats of both strains between 7 weeks and 6 months of age were used.

Immunization

Bovine S-antigen was provided by Drs. Magda El-Saied and Hitoshi Shichi (NEI) and Dr. Waldon B. Wacker (University of Louisville), and prepared as described by Wacker et al.10 The antigen was emulsified (1:1) in complete Freund's adjuvant (CFA) (GIBCO,
Table 1. Involvement of the pineal gland in actively immunized rats

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Mode of immunization</th>
<th>Pathologic changes</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Eye</td>
</tr>
<tr>
<td>Lewis</td>
<td>S-antigen in CFA</td>
<td>12/12</td>
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<tr>
<td></td>
<td>+ B. pertussis</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>PBS in CFA</td>
<td>0/4</td>
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<tr>
<td></td>
<td>MBP* in CFA + B. pertussis</td>
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</tr>
<tr>
<td>AVN</td>
<td>S-antigen in CFA</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>S-antigen in CFA + B. pertussis</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>MBP* in CFA + B. pertussis</td>
<td>0/6</td>
</tr>
</tbody>
</table>

* All rats of this group showed severe experimental allergic encephalomyelitis (EAE).
† None of these animals developed EAE.

Abbreviations: CFA, complete Freund's adjuvant; PBS, phosphate-buffered saline; MBP, myelin basic protein.

Grand Island, NY), enriched with Mycobacterium tuberculosis H37Ra to a concentration of 2.5 mg/ml. As a control for S-antigen, Dulbecco's phosphate-buffered saline (PBS) and myelin basic protein (MBP) from guinea pigs were used. The MBP was given by Dr. Bernard F. Driscoll (National Institute of Mental Health). A total volume of 0.1 ml/rat, containing 30-50 µg of S-antigen or 50 µg of MBP, was injected into the hind footpad of rats. In some experiments, an additional bacterial adjuvant, Bordetella pertussis, 10^10 bacteria per rat, was injected intravenously along with aforementioned immunization. The B. pertussis was of batch C-507F, gifts from Dr. Dale McFarlin (National Institutes of Health) and Dr. Scott Linthicum (University of Southern California).

Adoptive Transfer of EAU

The spleen and draining lymph node were collected from donor Lewis rats 12 or 13 days after immunization.
Fig. 2. Pathologic changes in the eye of a Lewis rat immunized with S-antigen in CFA. The rat was killed on day 13 after immunization. Unlike the pineal gland, a heavy involvement of polymorphonuclear cells (P), in addition to the mononuclear infiltration (M), was found in the retina, especially in the visual cell layer (Hematoxylin and eosin, X375).

Evaluation of EAU and Pineal Gland Involvement

Clinical signs of EAU were monitored daily, with or without a slit-lamp microscope. Actively immunized rats were killed usually between days 14 and 19 postimmunization. Recipient rats were killed between days 6 and 9 postinjection of the cultured cells. Eyes and pineal glands were fixed in 2.5% glutaraldehyde and embedded in glycol methacrylate. Sections cut at 3 μm were stained with toluidine blue or hematoxylin and eosin, and examined with a light microscope.

Results

Table 1 summarizes the data concerning the involvement of the pineal gland in Lewis or AVN rats immunized with S-antigen or control antigens. All 12 of the Lewis rats that were tested developed EAU clinically as well as histologically at day 12 to day 19 postimmunization with S-antigen emulsified in CFA. Four of these 12 Lewis rats showed cell infiltration in the pineal gland. Among the 12 rats, three rats were killed on day 12 after immunization when clinical signs of ocular inflammation first were observed. One of these rats already had cell infiltration in the pineal gland. The cell infiltration was moderate and located mainly at the peripheral or subcapsular area of the pineal gland (Fig. 1). The infiltrating cells were mono-
nuclear, small, and round in shape, and considered to be mostly lymphocytes. Unlike the pineal glands, a heavy involvement of polymorphonuclear cells (PMNs), in addition to the mononuclear infiltration, was found in the eyes of the same rats (Fig. 2). The Lewis rats treated with *B. pertussis* in addition to the S-antigen immunization (*n* = 5) developed more severe EAU as well as an earlier onset of the disease than rats immunized with S-antigen alone (11–15 days vs. 12–19 days). In addition, all rats in this group showed lymphocytic infiltration in the pineal gland, with many cells invading as far as the central area of the gland (Fig. 3). In contrast, control Lewis rats immunized with either PBS (*n* = 4) or MBP (*n* = 4) along with *B. pertussis* did not develop EAU, and no lymphocytic infiltration was found in their glands. All rats immunized with MBP showed experimental autoimmune encephalitis (EAE) and lymphocytic infiltration in the brain tissue.

In AVN rats, none of the three immunized with S-antigen in CFA showed EAU or any change in the pineal gland. On the other hand, all six AVN rats immunized with S-antigen with the additional injection of *B. pertussis* developed EAU and lymphocytic infiltration in their pineal glands. The histologic changes were similar to those found in the Lewis rats without *B. pertussis* treatment (see above). Again, control immunization with MBP in CFA along with *B. pertussis* injection did not induce any pathologic changes in both the eyes and the pineal gland (*n* = 6).

Table 2 summarizes the results concerning the involvement of the pineal gland in transferred EAU. In preliminary experiments, recipients of uncultured lymphocytes from immunized donors showed neither EAU nor pineal inflammation, even with high numbers of cells (10⁷–10⁸ cells per recipient). However, once lymphocytes were stimulated in culture with either Con A or S-antigen, EAU and pineal infiltration were found in recipients of even lower numbers of cells (Table 2). As few as 5 × 10⁶ lymph node cells cultured with S-antigen were reproducibly capable of transferring EAU to naive Lewis rats. EAU developed in recipient rats faster than in actively immunized rats (5–9 days vs. 12–19 days), and the clinical and histologic changes of EAU in recipients resembled those in actively immunized rats. Lymphocytic infiltration of the pineal gland was observed in three of the four recipients of 20 to 60 × 10⁶ lymph node cells cultured with S-antigen, but in none of the three recipients of a low number of cells (5 to 10 × 10⁶ cells/rat). The two
Fig. 4. The pineal gland of a Lewis rat with passively transferred EAU. The rat was injected with $90 \times 10^6$ spleen cells from a S-antigen-immunized donor that were stimulated in culture with concanavalin A. The recipient rat was killed eight days after cell transfer. Mild lymphocytic infiltration is seen in the peripheral zone of the gland (Hematoxylin and eosin, X80).

Discussion

The present study shows that rats that develop EAU after immunization with S-antigen also may develop pathologic changes in their pineal glands. The pineal changes were found to be antigen-specific, since no such changes were detected in control rats injected with CFA alone, or with the encephalitogenic antigen MBP. The close relationship between the ocular and pineal involvement was depicted further by the finding that the frequency and severity of pathologic changes in the two organs were affected similarly by the genetic makeup of the immunized rats and by the mode of immunization. Thus, pineal involvement was found in Lewis rats that are "high responders" for EAU, but not in the AVN rats that are "low responders" and did not develop EAU in this study following immunization with S-antigen and CFA alone. The ocular and pineal involvements were enhanced remarkably by treating the immunized rats with *B. pertussis*: all S-antigen-immunized rats, of both strains, developed both pineal and ocular changes following treatment with these bacteria. The mode of action of *B. pertussis* on disease induction is not clear, but accumulating evidence supports the notion that these bacteria increase the reaginic immunity and the activity of the mast cell system, and consequently, the permeability of the blood-organ barrier. The close relationship between the ocular and pineal involvement also was suggested by the findings that the inflammation in the pineal gland developed with kinetics similar to that of actively immunized Lewis rats without *B. pertussis* treatment.

Cellular infiltration in the pineal gland also was found in rats in which EAU was induced adoptively by the transfer of lymphocytes from S-antigen-immunized donors. This finding further supports the notion that the changes in both the retina and pineal gland are produced by the same immune-mediated mechanism. Moreover, this finding indicates that spe-
specifically sensitized lymphocytes, rather than antibodies, play the major pathogenic role in the disease induction in both the eye and pineal gland. Indeed, no antibodies to S-antigen were detected in sera of rats that were recipients of Con-A-stimulated spleen cells (unpublished data). Thus, it may be suggested that humoral immunity, whenever developed, plays merely an accessory role in the pathogenesis of the pathologic ocular or pineal changes.

The cells infiltrating the affected pineal glands were found to be lymphocytes, both in recipient rats and those actively immunized with S-antigen (Figs. 1, 3, 4). On the other hand, heavy involvement of PMNs was found in the eyes of the same rats (Fig. 2). The difference between the two organs is not clear and may be attributed to different factors such as (1) the immune response in the eye may be more intense (resulting from the presence of more S-antigen) and thus, the mediated inflammation may be more "acute" and favorable to PMN involvement; or (2) hypothetically, PMNs may be attracted to the eye by certain ocular components with selective chemotactic activity for PMNs.

Our findings differ from those of Wacker et al. in that the frequency of pineal involvement in the recipient rats was considerably lower than that of the ocular involvement; the mentioned authors observed more pineal than ocular involvement in recipient guinea pigs. The difference between the two animal species is not clear, but could be related to anatomic differences between the two species; unlike the rat, the guinea pig lacks retinal blood vessels. It is worth noting that less pineal than ocular involvement also was found in rats actively immunized with S-antigen (Table 1).

Key words: experimental autoimmune uveitis (EAU), retinal S-antigen, pineal gland, inflammatory changes, Bordetella pertussis, adoptive transfer of EAU

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