Allergic Adrenitis and Adenohypophysitis: Further Observations on Production and Passive Transfer

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ABSTRACT. Allergic adrenalitis and adenohypophysitis in rats are useful experimental models for the study of auto-immune injury to adrenal and pituitary glands. They were produced by injections of the corresponding homologous tissues with the aid of Freund's adjuvant. Heterologous tissues had little or no effect except for guinea pig pituitary. Pertussis vaccine was a useful adjuvant for adrenalitis but not for adenohypophysitis. Adrenals from neonatal rats were equal to or better than adult adrenal in antigenic potency. This observation suggested that steroid content was not a major factor in elicitation of adrenalitis. The atrophic adrenals from hypophysectomized rats had reduced potency for production of adrenalitis. The absence of the medulla in enucleated or transplanted glands did not prevent regenerated adrenal tissue from acting either as antigen or target for allergic adrenalitis. Adrenals was produced both by active immunization with adrenal antigen and adjuvants, and by passive transfer of living lymphoid cells from actively immunized donors to unimmunized recipients. Various strains of rats differed in susceptibility. The success of passive transfer depended on the histocompatibility of donor and recipient strains, and had no relation to the recipient strain's susceptibility to active immunization. Rats bearing transplantable hormone-secreting pituitary tumors were not susceptible to induction of allergic adrenalitis. (Endocrinology 84: 469, 1969)

AUTO-IMMUNE injury may be the cause of certain diseases of adrenal and pituitary glands in man (1–4). Allergic adrenalitis has been produced experimentally in guinea pigs (5–9), rabbits (7–10) and rats (11–14). Allergic adenohypophysitis is probably analogous to adrenalitis but has been produced only in rats (15). In lesions of both experimental diseases, light and electron microscopic (Hirano, Levine, Hoeneg and Ghatak, in preparation) studies have revealed mononuclear inflammatory cell infiltrates with or without confluence and necrosis (Fig. 1–3). There is no evidence as yet that humoral antiadrenal antibodies cause adrenalitis, but the pathogenetic significance of immunized lymphoid cells has been proven by passive transfer with lymph node suspensions (11, 12). The present study concerns the active and passive production of adrenalitis in several strains of rats with antigens of various sources, and related data on adenohypophysitis.

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Materials and Methods

Almost all active immunization experiments were done on inbred Lewis rats of either sex, 8–12 weeks old, obtained from Microbiological Associates, Inc., and maintained on Purina Laboratory Chow and tap water. The tissue antigens were obtained from freshly killed animals and human autopsies. No precautions were taken to reduce stress at the time of sacrifice. Fat was removed from the adrenals and posterior lobes from the pituitaries before freezing. The tissues were thawed, chopped, strained through a metal sieve and suspended in saline at a concentration of 40% (moist weight by final volume). The suspension was homogenized by cycling between 2 syringes connected with a 20-gauge double-lubed needle. The homogenate was emulsified in an equal volume of Freund's adjuvant, either "complete" (with killed tubercle bacilli) or "incomplete" (no tubercle bacilli) (11, 12). The 0.05 ml of emulsate injected intradermally into 1 of the right hind foot pads contained 10 mg (wet weight) of tissue. When indicated, 0.1 ml concentrated pertussis vaccine (about 20 billion organisms) was injected intradermally into the dorsum of the same foot at the same time for additional adjuvant effect (11). All injections were done with sterile precautions through 25-gauge needles with the aid of ether anesthesia. Rats were killed for histologic study by exsanguination under ether anesthesia. Adrenals with severe inflammation were enlarged and

The early development and fine structure of allergic adrenalitis.

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Table 1. Heterologous antigens for production of allergic adrenitis and adenohypophysitis

<table>
<thead>
<tr>
<th>Antigen*</th>
<th>Adrenitis†</th>
<th>Adenohypophysitis‡</th>
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<tbody>
<tr>
<td></td>
<td>with pertussis‡</td>
<td>without pertussis</td>
</tr>
<tr>
<td>Human</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Cow</td>
<td>1/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Dog</td>
<td>4/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Cat</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Rabbit</td>
<td>9/16</td>
<td>0/4</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>2/12</td>
<td>0/4</td>
</tr>
<tr>
<td>Gerbil</td>
<td>2/14</td>
<td>0/4</td>
</tr>
<tr>
<td>Gerbil (neonatal)</td>
<td>0/6</td>
<td>0/4</td>
</tr>
</tbody>
</table>

* 40% homogenate of adrenal or pituitary tissue, emulsified in equal volume of Freund’s complete adjuvant.
† Numerators: number of rats with lesions (all lesions were mild except half of those caused by guinea pig pituitary). Denominators: total number of rats.
‡ 0.1 ml pertussis vaccine concentrate in dorsum of foot that received the antigen. Usually sacrificed 10 days after inoculation (all other rats sacrificed after 14 days).

Results and Discussion

Antigenic potency of heterologous adrenals and pituitaries. Adrenitis has been produced in guinea pigs and rabbits with adrenal tissue, the origin of which was autologous (same individual animal), homologous (same species) or heterologous (different species) (5–10). In Lewis rats, isologous (same inbred isogenic strain) or homologous adrenal tissue was effective, but a single heterologous adrenal tissue (guinea pig) was ineffective (11). Because of this apparent lack of reactivity to a heterologous antigen in rats, adrenal glands from several species were incorporated into adjuvants and assayed. Adrenitis was found in some of the rats after inoculation of dog or rabbit adrenal tissue and occasionally after cow, guinea pig or gerbil adrenal tissue (Table 1). However, the lesions were invariably mild (a few, small scattered inflammatory infiltrates in the cortex) and were found only in rats that received pertussis vaccine as an ancillary adjuvant. Despite the aid of pertussis vaccine, heterologous adrenal tissue was distinctly less effective in rats than isologous or homologous tissue.

Heterologous pituitary tissue derived from cow, human, dog and rabbit produced little or no adenohypophysitis, but guinea pig pituitary was effective (Table 1; Fig. 3). Employment of pertussis vaccine as an ancillary adjuvant was not helpful; nor was it helpful with homologous pituitary antigen in previous work (15). There is no obvious explanation for the differences between adrenitis and adenohypophysitis with respect to the adjuvant role of pertussis vaccine and effectiveness of tissue antigens derived from the guinea pig.

Antigenic potency of neonatal adrenals. The adrenal of the newborn rat appears well developed by histologic criteria, and Barnett et al. found that a rabbit antiadrenal serum reacted with newborn or fetal rabbit adrenal in complement fixation tests (9). Therefore, it was not surprising when a preliminary experiment revealed that adrenals taken from Lewis rats within one day of birth, injected with adjuvants, produced severe adrenitis. It was of interest to compare the antigenic potency of adult and neonatal adrenals. To facilitate comparison, less-than-optimum conditions were chosen deliberately: early sacrifice (8 days after inoculation); omission of pertussis vaccine; incomplete rather than complete Freund’s
adjuvant (Table 2). The results of three experiments involving a total of five comparisons indicated that neonatal adrenal was equal to or better than adult adrenal in antigenic potency. Inasmuch as neonatal adrenals are low in corticosteroid content (16), this finding makes quite unlikely the suggestion of Milcou et al. (10) that the hormones themselves are the antigens responsible for adrenalinis. On the contrary, it seems more likely that corticosteroids in the adrenals would reduce the tissue's antigenic potency because of their anti-inflammatory action. However, we found 1.2 and 31.8 \( \mu g \) corticosterone in each ml of neonatal and adult 40% adrenal homogenates, respectively, used for the second experiment in Table 2 [method II of Vernikos-Danellis et al. (17)]. This large difference in steroid content was found in the very experiment in which the neonatal and adult adrenal homogenate induced nearly the same degree of adrenalinis. Therefore, steroid content does not appear to be an important factor in antigenicity of whole adrenal tissue.

**Antigenic potency of atrophic adrenals.** Adrenals were obtained from 18 CFE female rats two weeks after hypophysectomy (moist weight 15.6 mg each adrenal), and from 9 normal control rats of the same strain and age (32.8 mg each adrenal). Homogenates were prepared, emulsified in Freund's *incomplete* adjuvant (in order to ensure an intermediate degree of adrenalinis) and injected along with pertussis vaccine into Lewis rats. All 12 recipients of normal adrenal tissue developed adrenalinis, but only five of 12 recipients of adrenal tissue from hypophysectomized rats had lesions. Therefore, the adrenals from hypophysectomized rats had reduced antigenic potency, but this could have been caused by depletion of any of the elements affected by the severe atrophy.

**Antigenic potency of regenerated adrenal tissue.** The histologic appearance of allergic adrenalinis in some experiments has suggested that the cortex is more likely than the medulla to be the site of the antigen responsible for production of adrenalinis (9, 11). Furthermore, antiadrenal serum stained the cortex but not the medulla in immunofluorescence test (9). To contribute evidence on this problem, ten rats were subjected to bilateral adrenal enucleation, a procedure that eliminates all or most medullary tissue (18). Three weeks later, the regenerated adrenals were obtained and frozen. Because of the small yield and difficulty in processing it, the
Table 3. Susceptibility of various strains of rats to adrenalitis induced by active or passive immunization

<table>
<thead>
<tr>
<th>Strain†</th>
<th>Active adrenalitis*</th>
<th>Passive adrenalitis†</th>
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<tr>
<td></td>
<td>Severe</td>
<td>Mild</td>
</tr>
<tr>
<td>Lewis</td>
<td>4/5</td>
<td>1/5</td>
</tr>
<tr>
<td>(Lewis × BN) F₁</td>
<td>1/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Fischer 344</td>
<td>4/5</td>
<td>1/5</td>
</tr>
<tr>
<td>BN</td>
<td>3/5</td>
<td></td>
</tr>
<tr>
<td>Wistar-Furth</td>
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</table>

* Sacrificed 14 days after inoculation with neonatal Lewis rat adrenal, Freund's complete adjuvant and pertussis vaccine.
† Sacrificed 7 days after thermal injury of left adrenal and 1 day after passive transfer of lymph node cells from female Lewis donors with actively induced adrenalitis. Lesions of passive adrenalitis in left adrenal only.
‡ Male rats, inbred strains, all from Microbiological Associates, Inc., except Wistar-Furth from A. R. Schmidt Co.

Homogenate was made only 10% in concentration. The final emulsion was made with Freund's incomplete adjuvant and two or four times the usual volume was injected, along with the usual dose of pertussis vaccine. Despite the use of incomplete adjuvant, the low concentration of adrenal antigen, and the absence or near-
absence of medullary tissue, three out of six rats developed adrenitis, albeit mild. In view of these results, it is very unlikely that the medulla could be the sole source of the adrenal antigen.

Adrenitis in regenerated adrenals. Further evidence for this conclusion was provided by the reverse experiment, in which the regenerated adrenal provided the target rather than the antigen. Twelve Lewis rats were given normal isologous adrenal homogenate with complete adjuvant and pertussis vaccine. Two or six days later, the left adrenal was enucleated in all, and, in addition, the right adrenal was removed from some. When sacrificed 13 days after inoculation, all the regenerating adrenals exhibited severe adrenitis; residual medullary tissue was not detected in the sections. Six control rats that had the enucleation procedure but no antigen-adjuvant inoculations revealed no inflammation in their regenerating adrenals.

In another experiment with similar purpose, two adrenals taken from Lewis rats on the day of birth were implanted beneath the renal capsule of each of eight adult isologous rats. One week later, four of these rats were immunized with adrenal homogenate, complete adjuvant and pertussis vaccine. The rats were sacrificed two weeks later. All the transplanted adrenals in immunized rats revealed extensive mononuclear cell infiltration (Fig. 1) even more severe than that which afflicted the host’s own adrenals. In the control animals, neither transplanted nor host adrenals had inflammation (Fig. 2). Of particular importance was the absence of medullary tissue in the regenerated transplanted glands; presumably it was lost in the initial necrosis which affects all but the periphery of transplanted glands (Fig. 1, 2). It was concluded that removal of medullary tissue did not prevent adrenal tissue from acting either as antigen or as target for allergic adrenitis.

Adrenitis in various strains of rats. Adrenitis has been reported previously in Lewis and (Lewis X BN) F₁ hybrid rats (11). The response of these two strains was compared to three additional strains after immunization with Lewis rat adrenal homogenate and adjuvants (Table 3). Severe adrenitis was produced in the BN strain. Although adrenitis was observed in Fischer 344 and Wistar-Furth rats, their susceptibility was distinctly less.

Passive transfer of adrenitis in histocompatible strains. Adrenitis, like other types of delayed hypersensitivity, has been transferred passively with lymphoid cells from actively immunized donors to normal
recipients (11). Transfer with lymph node (12) or blood cells (13) was facilitated and accelerated by production of a zone of thermal coagulation necrosis with early reactive changes in one of the recipient's adrenals. The success of passive transfer probably depended on the survival of the donor cells in the recipient (19). In previous experiments (11–13), survival was ensured by employing inbred, isogenic, histocompatible animals as donors and recipients.

In the present work, the donors were 16 female Lewis rats, immunized with neonatal Lewis adrenal tissue, Freund's complete adjuvant and pertussis vaccine. Eight days later, the donors were killed, and the right popliteal, lumbar, sacral, renal, inguinal and axillary nodes draining sites of inoculation were removed and quickly processed into a cell suspension (11, 12, 20). The suspension was injected intravenously into 16 male recipient rats of various strains that had thermal injuries of the left adrenal inflicted seven days earlier with an electric soldering iron (12). The recipients were sacrificed 24 hours after transfer and the left adrenal was studied histologically for inflammation adjacent to the zone of thermal coagulation necrosis.

Lewis donor cells produced adrenalitis in Lewis, (Lewis X BN) F1 hybrid and Fischer 344 recipients but not in BN rats (Table 3). These results were readily explicable. Lewis rats were isogenic and therefore Lewis recipients were histocompatible with the donor cells. (Lewis X BN) hybrid recipients found nothing foreign in Lewis donor cells and were, therefore, genetically tolerant of them. Even Fischer 344 recipients were tolerant of Lewis donor cells for the brief duration of these transfer experiments because these two strains differ only in minor histocompatibility antigens (21). Only the BN recipients differed from Lewis in major histocompatibility antigens, and, therefore, rejected the transferred Lewis lymph node cells before adrenalitis could develop. These results were in accord with previous experiments of similar design on transfer of allergic encephalomyelitis (20), and confirm that histocompatibility of donor and recipient is essential for passive transfer with lymphoid cells. The requirement for histocompatibility, as well as the brief incubation period, eliminated the possibility that passive transfer of adrenalitis was some special, occult form of active immunization. It was noteworthy that susceptibility to passive transfer among the several strains bore no relation to their sensitivity to active immunization.

Adrenalitis in tumor-bearing rats. Fischer 344 rats bearing the transplantable MtT/F4 pituitary tumor have large adrenals, probably because of ACTH production by the tumor (22). Wistar-Furth rats carrying the transplantable MtT/W15 pituitary tumor have large adrenals, probably because of somatotrophic hormone production by the tumor (22). Seven tumor-bearing rats of each of these types were inoculated with neonatal Lewis adrenal antigen, Freund's complete adjuvant and pertussis vaccine. No adrenalitis was found in any of these animals although the adrenals were enlarged to 0.45–0.69 g/pair in Fischer 344 rats and 0.12–0.19 g/pair in Wistar-Furth rats. The absence of adrenalitis was not caused by lack of antigen in the enlarged glands, because similar enlarged adrenals proved capable of inducing adrenalitis in normal Lewis rats when homogenized and inoculated with complete adjuvant and pertussis vaccine. Rather, the absence of adrenalitis in MtT/F4 tumor-bearing rats was due to failure of immunization, inasmuch as the inoculation sites and draining lymph nodes had virtually no inflammatory reaction. Presumably, this was the result of hypersecretion of anti-inflammatory corticosteroids. The MtT/W15 tumor-bearing rats did not show such an obvious anti-inflammatory effect, but the control Wistar-Furth rats exhibited so low a susceptibility to adrenalitis (Table 3) that a minor steroidal
excess might have been sufficient to achieve the same effect.

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8. Terplan, K., E. Witebsky, and F. Milgrom, Arch Path (Chicago) 76: 333, 1963.