DEMONSTRATION IN VITRO OF DELAYED HYPERSENSITIVITY IN EXPERIMENTAL ALLERGIC ORCHITIS IN GUINEA-PIGS

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Summary. Cells in the peritoneal exudate obtained from guinea-pigs within 7 days of sensitization with testicular homogenate emulsified in complete Freund’s adjuvant, showed inhibition of migration when cultured in the presence of the supernatant from the testis homogenate. Simultaneously, the first signs of orchitis were observed. Delayed skin tests became positive only 19 days after sensitization when the orchitis was fully developed. Intertubular infiltration was found in few animals without any apparent relationship to the lesions of the seminiferous tubules. Anaphylactic antibodies were detected within 33 days of sensitization as shown by anaphylactic skin reaction and passive cutaneous anaphylaxis. The macrophage migration test appears to detect delayed hypersensitivity earlier than the delayed skin reaction in the experimental conditions used.

INTRODUCTION

Experimental allergic orchitis (EAO) can readily be produced in guinea-pigs and other species by injection of testicular homogenate in complete Freund’s adjuvant.

There are considerable data available to suggest that mechanisms of delayed hypersensitivity may be involved in the pathogenesis (Waksman, 1959). These include the fact: (a) that complete adjuvant is usually needed to induce the condition (Voisin, Delaunay & Barber, 1951; Freund, Lipton & Thompson, 1953, 1955; Brown, Glynn & Holborow, 1967), (b) that EAO can be successfully induced in normal animals by the transfer of lymphoid cells from affected animals (Stone, Lerner & Goode, 1968), and (c) that a delayed skin reaction is present (Broughton, 1962; Laurence, Carpuk & Perlbachs, 1965). The inability of serum containing antibody to transfer the disease (Mancini, 1964; Levaditi, Eyquem & Nazemoff, 1966) provides further proof that the sensitized cells themselves could be directly involved in the process of germinal cell destruction.

The inhibition of macrophage migration technique (George & Vaughan, 1962) is based on the ability of the antigen to prevent the migration of macrophages obtained from the peritoneal exudate of sensitized animals. The method
detects delayed hypersensitivity in a similar way to the delayed skin reaction, and has two important advantages: (1) the ability to reproduce the results regardless of skin reactivity and (2) the possibility of obtaining quantitative data. The technique was applied here to study the contributory rôle of the delayed hypersensitivity in the pathogenesis of EAO.

MATERIALS AND METHODS

Preparation of testicular homogenate

Guinea-pig testes were homogenized with an equal volume of saline in a high-speed homogenizer. Each animal received 250 mg wet weight of testes.

Sensitization of animals

Random-bred male guinea-pigs weighing 350 to 500 g were used. Sensitized animals were divided into four groups. Group 1: Fourteen animals received 250 mg of testicular homogenate emulsified in complete Freund’s adjuvant, with 100 µg of tubercle bacilli H37 Ra (Difco). Group 2: Five animals received 250 mg of testicular homogenate alone. Group 3: Five animals received 250 mg of homogenate of homologue thyroid gland emulsified in complete Freund’s adjuvant. Group 4: Five animals received complete Freund’s adjuvant alone.

Animals were sensitized by injecting a single dose of the antigen into several places on the back, which had been previously prepared by shaving. Every week two animals in Group 1 and one animal in Groups 2, 3 and 4 were killed. Skin tests were made by intradermal inoculation into the shaved flank of supernatant from testicular homogenate (2-2 mg protein by the method of Lowry, Rosebrough, Farr & Randall (1951) in saline. Recordings were made 1 hr later of any anaphylactic skin reaction and 24 hr later of any delayed skin reaction.

Histological examination

The skin at the site of the skin test, and the testes and epididymides of the animals, were studied microscopically. Tissues were fixed in Bouin’s fluid and embedded in paraffin wax. Sections of 4 to 6 µm were stained with haematoxylin and eosin.

Macrophage migration test

Peritoneal macrophage cells were induced by the injection of 20 ml of paraffin oil. Four days later, the exudate cells were harvested and washed. Cell concentration was adjusted to $6 \times 10^8$ cells/ml. Capillary tubes were filled with the cell suspension and sealed at one extremity. After centrifugation (1000 rev/min for 3 min) the tubes were cut at the cell-fluid interface. The part of the tube containing the packed cells was placed in Mackaness-type chambers of 1.2 ml capacity, two tubes per chamber. Sufficient material was obtained from each animal to fill eight or ten capillary tubes; the cells in half the tubes migrated in chambers filled with Eagle’s minimal tissue culture medium with 20% calf serum, while the cells in the remaining half migrated in chambers filled with the same media plus the supernatant of testicular homogenate (110 µg protein/ml). The chambers were then incubated for 24 hr at 37°C. During this
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time, the cells migrated out of the tubes and on to the glass. The area of migration was afterwards projected, drawn and weighed. The following formula was used:

\[
100 - \frac{\text{Area of migration with antigen}}{\text{Area of migration without antigen}} \times 100
\]

= % inhibition of migration with antigen

Table 1

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Days</th>
<th>G-pig No.</th>
<th>Skin test</th>
<th>Inhibition of migration of macrophage†</th>
<th>% Semi-niferous tubules damaged</th>
<th>Passive cutaneous anaphylaxis</th>
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<tr>
<td></td>
<td></td>
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<td>1 hr and 24 hr</td>
<td>Mean ± S.D.</td>
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<td>Macrosc.</td>
<td>Microsc.*</td>
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<td>5</td>
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<td>5</td>
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<td>Complete Freund's adjuvant (Group 4)</td>
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* Degrees of mononuclear infiltration in dermis and perivascular areas.
† Average and standard deviation of percentage inhibition of migration. This average represents several experiments per animal (eight to ten tubes).

The average from several experiments per animal (eight to ten tubes) was calculated and expressed as the percentage inhibition of migration. Table 1 shows the mean and standard deviation of these data. Inhibition levels of 25% or more were found to be significant in our experimental conditions and indicated the presence of delayed hypersensitivity.
Passive cutaneous anaphylaxis

Guinea-pig sera were diluted serially with saline and 0.1 ml doses were injected into the abdomen which had previously been shaved and marked indelibly. After 4 hr, supernatant from the testicular homogenate (5 mg protein) together with a 2% solution of Evans blue were given intravenously. The animals were killed 20 min later and the skin opened so that the lesions could be evaluated (Weir, 1967).

RESULTS

Seven days after animals were injected with testicular homogenate and complete Freund's adjuvant the germinal epithelium showed some damage in 25% of seminiferous tubules (see Table 1). At the same time, a significant inhibition of macrophage migration was found and remained until the end of the experiment (41 days). The delayed skin reaction did not appear until the 19th day after sensitization. A papule was observed in all animals after a further 24 hr.

Histologically, this showed a lymphocytic infiltration around the small vessels. At the same time, more extended testicular lesions were found in approximately 80% of the seminiferous tubules (Text-fig. 1).

Anaphylactic skin reactions appeared 1 hr after testing, in animals sensitized 33 days before. This reaction disappeared 4 hr later and, after 24 hr, a delayed skin reaction appeared at the same place. Positive passive cutaneous anaphylaxis was also obtained with sera from these animals. The immunological response (anaphylactic skin reaction, delayed skin reaction and inhibition of macrophage migration) was found associated with testicular damage from 33
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Days until the end of the experiment. An intertubular mononuclear cell infiltration was only seen in some animals.

The control group which was injected with testicular homogenate alone, showed neither immunological response nor testicular lesions, with the exception of two animals in which a delayed skin reaction was detected 20 days after sensitization. The control group also showed less marked testicular lesions (15% of the seminiferous tubules were affected) and only 20% inhibition of peritoneal macrophage cell migration.

The control group sensitized with thyroid homogenate and complete Freund’s adjuvant also showed neither immunological response nor testicular lesions. Mononuclear cell infiltration in the interfollicular areas of the thyroid gland was found 20 days after sensitization. This picture remained unchanged until the end of the experiment.

The control group which was injected with complete Freund’s adjuvant alone did not show any reactions.

DISCUSSION

In the present study, as in those by other authors, injections of testicular homogenate with complete Freund’s adjuvant were found to cause EAO and two types of immunological response in guinea-pigs: the presence of circulating antibodies and a delayed hypersensitivity. The first signs of mild orchitis were observed 7 days after sensitization. At that time, the delayed skin reaction was still negative, but macrophage migration was significantly inhibited.

When a positive delayed skin reaction was observed after 19 days, the testicular lesions affected 80% of the seminiferous tubules. In the present study, the time of appearance of the delayed skin reaction which differs from that reported by other authors (Brown et al., 1967; Voisin & Toullet, 1968), may have been due to a different skin reactivity, which is very common among different strains of guinea-pigs.

The intertubular infiltration, consisting of lymphocytes, reticulo-endothelial cells and some plasma cells, was found in only a few animals and bore no relationship to the degree of the lesions of the seminiferous tubules. This finding coincides with previously reported data (Freund et al., 1953; Brown, Glynn & Holborow, 1963) and points to the problem of the pathological significance of the intertubular cell infiltration in this experimentally induced condition.

It was also observed that an anaphylactic skin reaction and passive cutaneous anaphylaxis were detectable after 33 days of sensitization when orchitis was already fully developed. This apparent lack of correlation of circulating antibodies with the initiation of germinal cell damage is now being investigated in our laboratory.

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REFERENCES


