

ADRENALECTOMIZED MICE AS AN EXPERIMENTAL MODEL FOR THE STUDY OF THE BIOLOGICAL ACTIVITY OF IMMUNE COMPLEXES

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Local and systemic reactions to an immune complex formed in vitro can be obtained in adrenalectomized mice. The principles for the composition of the immune complexes giving the appearance of their general (toxic) action and their ability to evoke local reactions are established. Factors inhibiting the anaphylactic reaction (injection of cortisone, antihistamines, general anesthesia) weakened the reaction to the immune complex. The local reaction to the immune complex was diminished by an artificially induced fall in the blood complement level in the animals.

KEY WORDS: adrenalectomy; immune complex; hypersensitivity of immediate type.

Sensitivity to the general anaphylactic reaction under conditions of both active and passive sensitization is sharply increased in adrenalectomized mice [2-4, 6]. It is important to obtain information on the response of adrenalectomized mice to injection of immune complexes formed in vitro. According to data in the literature, the response to immune complexes is characterized as an allergic reaction of immediate type [6]. Cases already reported relate to experiments on guinea pigs and rabbits and, to a lesser degree, experiments on mice [1, 5].

The object of this investigation was to study whether general and local reactions to injection of immune complexes are more frequent in adrenalectomized than in intact mice and to assess the effect of certain factors modifying the manifestation of anaphylaxis on these reactions.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice weighing 12-14 g, into which immune complexes were injected 48 h after removal of both adrenals, and on normal mice for comparison.

Immune complexes from horse serum and corresponding mouse antisera were used. The complexes were formed between ingredients in different proportions: in the zone of equivalence, in an excess (2-8-32-fold) of antigen, and in an excess (4-16-fold) of antibodies. In every case the quantity of antiserum remained unchanged, but the quantity of added antigen was varied. The mixtures were kept for 2 h at 37°C and for 18 h at 4°C. With the proportions of ingredients used, no visible precipitation took place. The test mixtures were injected intradermally in a volume of 0.1 ml to reveal the local reaction, and intravenously in a dose of 1 ml to assess their general action. Systemic reactions were assessed by the number of animals dying from acute shock during the first 24 h and local reactions by the size of the "blue spot" on the inner aspect of a piece of skin dissected 30 min after intravenous injection of 0.25 ml of a 0.5% solution of Evans' Blue 2 h after injection of the complex.

The effect of the following factors on the general and local reactions was tested: injection of hydrocortisone and diphenhydramine, carrying out the tests on anesthetized animals and also on mice with an artificially lowered complement titer, and finally, by the use of animals immunized against the antigenic components of the complex.

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TABLE 1. Frequency of Local and Systemic Reactions to Immune Complexes of Varied Composition in Normal and Adrenalectomized Mice

Composition of complexes with respect to ratio between antigen and antibodies	Local reactions		Systemic reactions	
	normal mice	adrenalectomized mice	normal mice	adrenalectomized mice
Excess of antibodies (4-16-fold)	2/12	7/15	0/11	2/11
Zone of equivalence	0/6	2/7	0/5	1/5
Excess of antigen:				
2-fold	0/6	1/6	0/6	3/6
8-fold	0/6	8/10	0/5	9/13
32-fold	0/6	0/5	0/6	2/6
Total . . .	2/36 (5%)	18/43 (41%)	0/33	17/41 (41%)
Except experiments with immune complex with an eightfold excess of antigen	2/30 (6,6%) $P < 0,02$	10/33 (30,3%)	0/23 $P < 0,01$	8/28 (28,5%)

Legend. Here and in Tables 2 and 3, numerator shows number of animals giving a reaction (or dying); denominator shows total number of animals.

Hydrocortisone was injected subcutaneously in a dose of 3 mg and diphenhydramine intraperitoneally in a dose of 10 mg/kg body weight, 18 and 3 h before the experiment.

In the experiments with ether anesthesia the complexes were injected into animals while in a state of deep sleep, which continued for 30 min.

A considerable decrease in the complement titer of the experimental animals was produced by injecting "antimouse serum" (i.e., the serum of a rabbit immunized with a suspension of mouse spleen in mouse serum) in a dose of 0.05 ml into their blood stream 30 min before application of the immune complex. Numerous experiments in the writers' laboratory have shown that this procedure invariably leads after 15 min to a sharp fall in the complement titer, which thereafter remains at a low level (no higher than 10% of the initial) for 9-12 h.

Mice immune against the antigenic component of the complex were obtained by immunization intravenously with horse serum in doses of 0.1 and 0.25 ml at an interval of 3 days, followed by revaccination 1 month later in a dose of 0.05 ml.

EXPERIMENTAL RESULTS

As Table 1 shows, intravenous injection of the immune complex did not cause death of any of the group of normal mice but caused death of 17 of the 41 adrenalectomized animals (41%). Local reactions were observed with the same frequency in the adrenalectomized animals to intradermal injection of the immune complexes (in 18 to 43 mice, i.e., in 41%), whereas in the normal mice there were only isolated positive tests (in 2 of 36 animals, i.e., 5.5%).

Both the number of deaths among the mice and the frequency of the local reactions increased most substantially (up to 70-80%) in experiments with immune complexes formed to an eightfold excess of antigen. However, even if the results of this most demonstrative group of animals (and the corresponding controls) were disregarded, the differences between the adrenalectomized and intact animals still remained distinct and significant (Table 1).

The effect of the various factors listed above on the systemic and local reactions was studied under conditions of maximal severity of the reactions, i.e., after injection of immune complexes containing an eightfold excess of antigen. As will be clear from Table 2, preliminary injection of cortisone or diphenhydramine into adrenalectomized mice, and also deep ether anesthesia, either completely prevented or sharply reduced ($P < 0.05$) the mortality among the animals after injection of the immune complex.

A sharp fall in the complement titer had a similar action on the local reaction.

The effect of the immunological status of the experimental mice, i.e., their sensitization to the antigenic components of the immune complex, on the reactions under investigation was studied with the use of complexes of low activity, namely: in the zone of equivalence and with a small and large excess of antigen. It was expected that under these conditions the complexes would be more active. This was so as regards general toxicity

TABLE 2. Effect of Certain Drugs, of Ether Anesthesia, and of a Sharp Fall in the Complement Titer on Death of Mice and Frequency of Local Reactions to Injection of Immune Complex into Adrenalectomized Mice

Character of test	Death of mice	Local reactions
Preliminary injection of cortisone (3 mg, twice)	0/13	0/6
Preliminary injection of diphenhydramine (10 mg/kg, twice)	3/10 (30%)	Not stable
Ether anesthesia	3/10 (30%)	"
Sharp fall in complement titer	Not stable	0/6
Control (adrenalectomized mice without additional treatment)	9/13 (69%)	8/10

TABLE 3. Effect of Preliminary Immunization of Mice with the Antigen of the Complex on their Sensitivity after Adrenalectomy to the Immune Complex

Nature of complexes tested	Death of animals		Local reactions	
	experiments (sensitized to antigen of complex)	control (not sensitized to antigen of complex)	experiments (sensitized to antigen of complex)	control (not sensitized to antigen of complex)
In zone of equivalence	4/4	1/5	3/6	2/7
Antigen in excess				
2-fold	3/4	3/6	0/7	1/6
32-fold	4/4	2/6	2/6	0/5
Total . . .	11/12 (98%)	6/17 (35%)	5/19 (27%)	3/18 (17%)

(Table 3). Of the 12 mice previously immunized with the antigen of the complex 11 (92%) died, whereas under analogous conditions only 6 of the 17 (35%) adrenalectomized animals receiving no further treatment died ($P < 0.01$). However, preparation of the animals in this way did not affect the frequency of the local reactions.

The results described above show that bilateral adrenalectomy considerably increases the sensitivity of mice to general and local administration of immune complexes. This suggests that this experimental model is suitable for the detection of circulating immune complexes in the experimental and clinical analysis of the corresponding pathological processes. Some special features of the conditions for detection of the general and local reactions merit attention: Both reactions were observed most frequently in experiments with immune complexes containing a small excess of the antigen; the local reaction also frequently appeared in response to the complex with a small excess of antibodies, possibly because of the very small aggregates formed; the frequency of the systemic reaction was considerably increased if the tests were carried out on animals sensitized to the antigen of the complex.

Meanwhile the results can also shed light on the character of reaction to immune complexes. In fact, the increased frequency of the general and local reactions in adrenalectomized animals and the decrease in the frequency of general reactions under the influence of cortisone, antihistamines, and general anesthesia all signify that the reactions are anaphylactic in nature and can be classed as allergic reactions of immediate type. Abolition of the local reactions by an artificial lowering of the complement level in the experimental animals indicated that complement is confirmed in the mechanism of these reactions.

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