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INFLUENCE OF ANTI-LYMPHOCYTE SERUM ON SKIN HISTAMINE IN MICE*

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ABSTRACT

Injections of anti-lymphocyte serum markedly reduced skin histamine in mice ten weeks after treatment. This decrease is compared with the similar decrease found after treatment with glucocorticoids. Although the effect may simply reflect a rather general action on body processes, it is suggested that these immunosuppressive agents may in part act by their effects on histamine.

There is still no general agreement on the nature of the properties to which antilymphocytic serum (ALS) owes its power. Although lymphocytes are probably the targets of its action (1, 2), ALS does not act through lymphocyte depletion (3). ALS is especially effective in the homograft reaction and other types of delayed hypersensitivities. Delayed allergic reactions are generally not believed to be due to histamine release. However, the participation of histamine in these reactions cannot be ruled out (4). Recent studies by Moore and co-workers (5–7) demonstrated a 33-fold elevation of histidine decarboxylase activity and a peak increase of urinary histamine in homografting at the time of rejection together with a marked reduction in rejection by histidine decarboxylase inhibitors. Histamine is formed by enzymatic decarboxylation of histidine. Furthermore, Inderbitzin (8), in his early studies on delayed hypersensitivity reactions of skin, found that ALS besides virtually abolishing these reactions, greatly diminished the local histamine increase normally observed (4, 9).

With the above in mind, we studied histamine in normal mouse skin following treatment with ALS.

MATERIALS AND METHODS

Anti-lymphocyte serum was prepared using the technique described by Gray (10). Thymuses and inguinal lymphnodes were excised from mice and the tissues pressed through stainless steel mesh into Hank's balanced salt solution. The resulting cell suspension consisting mainly of lymphoid cells was washed three times in normal saline resuspended in 5 ml saline and injected intravenously into the ear vein of a white rabbit weighing approximately 2.5 kg. Between 5×10^8 and 1×10^9 cells were injected. A similar injection was given 14 days later. One week after the second injection, the rabbit was exposed to a lethal dose of ether and blood was collected by cardiac puncture. The blood was allowed to coagulate, the serum removed, heated to 56° C for one-half hour, and absorbed with red blood cells from mice of the same strain. The leukocyte-agglutination titer, using thymus cells was 1:512. No such agglutination occurred with serum from untreated rabbits.

Ten mice weighing approximately 40 gm were injected subcutaneously with 0.2 ml anti-lymphocyte serum every other day up to a total dose of 2 ml. Ten weeks after the last injection the mice were sacrificed by ether and their normal back skin investigated for histamine. In addition, a control group of nine mice of identical ages were studied simultaneously. All mice were albino mice of strain Strit Leo.

Skin samples freed of panniculus carnosus and subcutaneous fat, weighing approximately 100 mg, were minced and lyophilized for 48 hours over phosphopentoxide at room temperature under a pressure of 2 mm Hg. Defatting was carried out by shaking twice with 15 ml ether through one hour. The dried and defatted samples were homogenized in 5 ml of a 0.4 N perchloric acid using a motordriven glass homogenizer. The homogenate was centrifuged, and a 4 ml aliquot of the supernatant was analyzed for histamine by the spectrofluorometric method of assay (11).

RESULTS

The results of the histamine determinations are summarized in Tables I and II. The histamine content of grossly normal back skin was reduced significantly ($P < 0.0025$) in mice treated with anti-lymphocytic serum. Skin histamine of non-treated mice ranged from 123.7 to 430.8 μg/g. This corresponds to the range of normal values for mouse skin found in previous studies by one of the authors (12). All histamine values are microgram histamine base per gram dried defatted skin.

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The general state of mice from both groups was good at the time of sacrifice, and on the whole, the experimental group showed no signs of toxicity from the anti-lymphocytic injections.

COMMENTS

Our results are in agreement with the data of Inderbitzin (8), who as far back as in 1956, reported that antiserum against lymphocytes reduced the local histamine increase found in delayed allergic reactions. He suggested that the reduced increase in skin histamine was due to a decreased amount of lymphocytes in the reacting area. In our hands, ALS reduced the content of histamine in grossly normal skin ten weeks after treatment. We did not study histological sections. It is, however, unlikely that the skin contained significant amounts of lymphocytes. Besides, lymphocytes are not rich in histamine (13). On the basis of the present study, we are not prepared to discuss the mechanisms of the reduction of skin histamine following ALS. It certainly is possible that the effect of ALS on skin histamine simply reflects a rather general action on body processes. A similar reduction in skin histamine has been found after glucocorticoids (14). In view of the present knowledge of increased histamine formation and accumulation in delayed allergic reactions (4, 6, 7), it is, however, tempting to suggest that ALS as well as glucocorticoids may in part act by their effect on histamine. This explanation is by no means contradictory to other proposed modes of action for ALS (15).

TABLE I

Skin histamine in grossly normal back skin of mice ten weeks after treatment with ten injections of 0.2 ml anti-lymphocyte serum

No.	μg histamine per g
1	75.3
2	122.7
3	154.6
4	84.4
5	76.7
6	130.9
7	105.8
8	133.9
9	76.3
10	79.8
Mean ± S.E.	104.1 ± 9.3

TABLE II

Histamine in skin from backs of control mice

No.	μg histamine per g
1	306.3
2	270.6
3	430.8
4	358.4
5	200.2
6	127.4
7	132.3
8	238.4
9	123.7
Mean ± S.E.	243.1 ± 36.3

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