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## SKIN HISTAMINE AND BASOPHILIC LEUKOCYTES IN THE NORMAL LYMPHOCYTE TRANSFER TEST

By

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Within recent years much attention has been paid to the search for simple methods of choosing human homograft donors. In 1963, the normal lymphocyte transfer test (N.L.T. test) was devised by *Brent & Medawar* (1) as a means of predicting the intensity of the reaction which a homograft would provoke in its recipient. These authors used guinea-pigs and expected that the test might be applicable to man. Later, *Gray & Russel* (3) demonstrated that intradermal injections of homologous lymphocytes in humans produced a characteristic skin reaction after 24 to 48 hours, while autologous lymphocytes produced no similar reaction. By the "skin-window" technic (4), *Wolf-Jürgensen & Schwartz* (8) revealed a basophilia in response to homologous, but not to autologous, lymphocytes. The presence of basophils in the cellular exudate of "skin-window" seems to be specific for the delayed-type of hypersensitivity reactions (6), and "skin-window" examination has been suggested important in the quantitative evaluation of the N.L.T. test (8).

Analysis of skin histamine in experimental allergic contact dermatitis and primary irritant dermatitis (9) have suggested

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that histamine plays a part in delayed cutaneous hypersensitivity reactions, and that a significant higher histamine level is specifically associated with allergic response in patch tests. The present study was undertaken to investigate whether the transplantation of homologous lymphocytes would induce an increase in skin histamine levels as well as basophilia. The determinations were carried out by the spectrofluorometric assay method devised by *Shore et al.* (5).

## MATERIAL AND METHODS

The studies included 12 persons aged 21 to 77 suffering from various local skin diseases not involving the skin of the arms. The total leucocyte and differential leucocyte counts of all patients were within normal limits.

Lymphocyte suspensions were prepared by the method described by *Brent & Medawar* (1). The suspension contained approximately 5 million lymphocytes per 0.1 ml. Injection of at least 3 million lymphocytes seems necessary to produce a maximum reaction (7). More than 95 per cent of the lymphocytes were intact at the time of injection. This was shown by the trypan-blue test.

The "skin-window" was made by scraping away the epidermis from a 1 square cm area of the forearm. From 0.1 to 0.2 ml cell suspension was injected directly in the area, homologous lymphocytes in the volar surface of one forearm, and autologous lymphocytes in a symmetrical site of the other. The excoriations were covered by coverslips, which were changed at every three hours for 48 hours. After removal, the coverslips were dried and stained by the May-Grünwald-Giemsa method. A differential count was made on all smears. At least 200 cells were counted in each.

Furthermore, from 0.1 to 0.2 ml of the same suspensions of homologous and autologous lymphocytes were injected intradermally at a distance of at least 5 cm from the corresponding "skin-window". The gross reactions were read after 48 hours, and samples of approximately 30 mg of skin were removed from the injection sites by the punch biopsy technic. Normal skin was taken for control at a distance of at least 5 cm from the site of injection of autologous lymphocytes. The subcutaneous fat was removed with knife or scissors, and the skin specimens were frozen at  $-20^{\circ}\text{C}$ , if not assayed immediately. Skin histamine was assayed spectrofluorometrically in dried and defatted skin according to a procedure previously described (9).

## RESULTS

In all patients, after forty-eight hours, intradermal injections of homologous lymphocytes produced an area of in-



TABLE 1

*Skin Histamine Levels, Maximum per cent of Basophils in Skin-Windows and Degree of Induration in Skin Injected with Autologous and Homologous Lymphocyte Suspensions.*

| Control skin histamine $\mu$ | Skin injected with      |                            |                   |                         |                            |                   |
|------------------------------|-------------------------|----------------------------|-------------------|-------------------------|----------------------------|-------------------|
|                              | Autologous lymphocytes  |                            |                   | Homologous lymphocytes  |                            |                   |
|                              | Degree of induration mm | Max. per cent of basophils | $\mu$ g histamine | Degree of induration mm | Max. per cent of basophils | $\mu$ g histamine |
| 30.6                         | 2                       | 0.5                        | 26.9              | 7                       | 3                          | 92.0              |
| 30.4                         | 0                       | 0                          | 29.6              | 10                      | 2.5                        | 33.9              |
| 25.6                         | 0                       | 0.5                        | 31.3              | 6                       | 3                          | 27.0              |
| 62.3                         | —                       | 0.5                        | 91.6              | —                       | 6                          | 107.8             |
| 32.2                         | 0                       | 1.5                        | 41.5              | 12                      | 4                          | 45.9              |
| 25.6                         | 0                       | 1.5                        | 36.6              | 5                       | 5                          | 99.5              |
| 12.9                         | 0                       | 0.5                        | 8.0               | 9                       | 2                          | 11.0              |
| 42.8                         | 0                       | 0                          | 55.5              | 6                       | 5                          | 66.5              |
| 18.0                         | 0                       | 0                          | 15.1              | 10                      | 3                          | 14.8              |
| 26.1                         | 4                       | 1                          | 21.4              | 10                      | 2                          | 41.0              |
| 34.8                         | 0                       | 0                          | 20.9              | 6                       | 2.5                        | 43.8              |

tions of autologous lymphocytes. One series of skin samples was lost during the technical procedure. The average rise in skin histamine was 22.0  $\mu$ g/g after homologous lymphocytes, and 3.4  $\mu$ g/g after autologous lymphocytes. In some cases the skin histamine increased to 3 or 4 times the normal values. Previous observations (9) of histamine in eleven different pieces of skin from the same area of the same individual showed a standard variation of  $\pm 5.8$   $\mu$ g/g. The standard error of the method itself was calculated to 4.5  $\mu$ g/g. All values represent  $\mu$ g histamine base per gram dried defatted skin.

#### DISCUSSION

The data of the investigation confirm that, although basophils are found in small numbers in "skin-windows" after injection of autologous lymphocytes in N.L.T. tests, a highly increased number of basophils is only observed after injection of homologous lymphocytes (Fig. 1). The data also show that

skin histamine increase is a frequent finding following transplantation of homologous lymphocytes, while it is relatively rare and not as high after transfer of autologous lymphocytes. In both cases there are considerable individual variations. High histamine values were seen in experiments with a large count of basophils in the cellular exudate (Table 1). This is in accordance with the concept of basophils as carriers of histamine. The increased skin histamine values and basophilia in the N.L.T. test support the notion that this inflammatory reaction is of the delayed hypersensitivity type. In addition to the visual assessment of the N.L.T. test and "skin-window" examination, determinations of skin histamine may possibly prove valuable in the quantitative evaluation of the response. Individuals reacting with a high skin-histamine increase should be expected to be unsuitable donors with strong transplantation antigens to the person supplying the lymphocytes of the test. Individuals reacting with a slight skin histamine increase or having normal skin histamine should be suitable donors.

It is concluded that skin histamine after transplantation of normal homologous lymphocytes in most cases is increased but as expected there is a considerable individual variation. Probably the lymphocytes only react against strong transplantation antigens which differ from those of the donor.

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