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EFFECT OF ANTILYMPHOCYTE SERUM ON THE CELLULAR EXUDATE IN SKIN WINDOWS

BY

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Lack of agreement still prevails concerning the precise mode of action of antilymphocyte serum (ALS). ALS is an antibody or complex of antibodies in the 7S group directed against antigens in or on, though not specific for, lymphocytes (Levey & Medawar 1966, 1967). Various methods have been used for the production of potent ALS (Monaco *et al.* 1967; Jooste *et al.* 1968), but as yet no satisfactory *in vivo* test for the immunosuppressive activity has been found. A number of parameters for measuring the activity of the serum are available, *e.g.* erythrocyte, lymphocyte and platelet agglutination, as well as lymphocyte cytotoxicity (Woodruff *et al.* 1967), opsonization of lymphocytes *in vitro* (Greaves *et al.* 1969) and rosette inhibition (Bach & Antoine 1968).

The aim of the present study was to determine, by means of the skin window technique, whether ALS has any influence on the unspecific cellular exudate, *e.g.* inhibition of the lymphocyte migration, and whether such an effect can be used in an *in vitro* test.

MATERIAL AND METHODS

Thirty-three patients admitted to the Department of Dermatology volunteered to participate in the study. None of them was suffering from any skin disease

on the forearms (where the skin windows are placed) or from any blood disease, nor were they subjected to internal treatment.

The skin window technique used was originally described by *Rebuck* (1940). The epidermis from an area of the forearm about 1 cm² in size was scraped off with a sterile knife. A sterile coverslip was placed on the eroded surface and affixed with tape. The slips were changed at three-hour intervals through 48 hours after which time they were stained by the May-Grünwald-Giemsa method, and differential counting was carried out.

Two simultaneous skin window tests were made on each person, one on each arm. On one arm, 1 mg anti-lymphocyte-serum-IgG (ALG) in 0.1–0.2 ml saline was injected intradermally into the excoriated area before the first coverslip was put in place. On the other arm the same amount of control serum with control gammaglobulin was injected. Only one injection of ALS was made in each test, and the amount injected was a minimal quantity compared with that used for intravenous immunosuppression.

Production of ALS and IgG (ALG). Various methods were used for production of the four sera mentioned below. Each serum was a pool consisting of sera from ten rabbits immunized with human lymphocytes isolated from the buffy coat of donor blood. The blood was drawn in citrate, centrifuged for 15 minutes at 1850 g, after which the buffy coat could be collected. After sedimentation of the erythrocytes with dextran, the supernatant was filtered through cottonwool, thus resulting in a pure lymphocyte suspension.

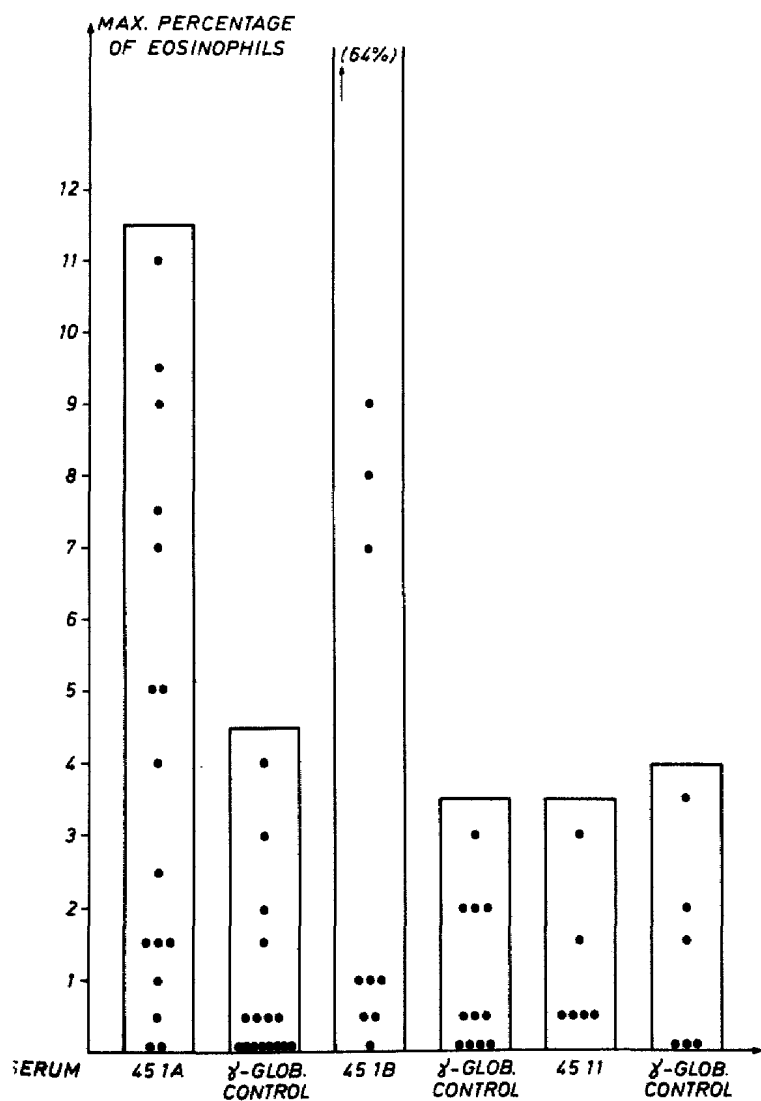
Crude gamma-globulins were produced by fractionated salting out with ammonium sulphate (50 per cent and 48 per cent), followed by dialysis. From that, non-specific proteins were separated in a milieu of barium salts by absorption with bentonite to the precipitation point, after which the IgG, thus purified, was obtained by salting out with 50 per cent ammonium sulphate, followed by dialysis. The IgG was freeze-dried in nitrogen atmosphere and stored in sealed ampoules.

1) Serum 45 I A was produced by subcutaneous injection of 1.4×10^8 lymphocytes in Freund's adjuvant. After one month 162.5 ml pooled serum was collected and 4.7 mg IgG/ml serum recovered. The cytotoxic titre of this serum was relatively high, viz. 1:64. Agglutination titre 1:64.

2) Serum 45 I B was produced from the same rabbits as 45 I A, i.e. by subcutaneous injection of 1.4×10^8 human lymphocytes in Freund's adjuvant. Two months after injection of the cells, a total of 145 ml of serum was col-

Fig. 1.

Graphic presentation of the maximum eosinophile percentage in skin windows. Each dot represents the preparation in a full skin window experiment where the largest number of eosinophils can be demonstrated.



lected with 8.2 mg IgG/ml serum. The cytotoxic titre was low, *viz.* 1:8. Agglutination titre 1:128.

3) Serum 45 II was produced by two intravenous injections at intervals of two weeks of 1.3×10^8 and 1.5×10^8 lymphocytes, respectively. In this case 211 ml serum was obtained with 4.7 mg IgG/ml serum. Cytotoxic titre 1:256. Agglutination titre 1:256.

4) Serum 45 III was produced by three intravenous injections of 1.8×10^8 , 2.4×10^8 and 1.3×10^8 lymphocytes. The first and second injections were given with an interval of 18 days and the third injection after a further 56 days. Amount of serum 114 ml with 8.6 mg IgG/ml. Agglutination titre 1:128. In some cases this serum (cytotoxic titre 1:64) caused local erythema in the skin and was therefore omitted from the study.

All sera were pyrogen-free.

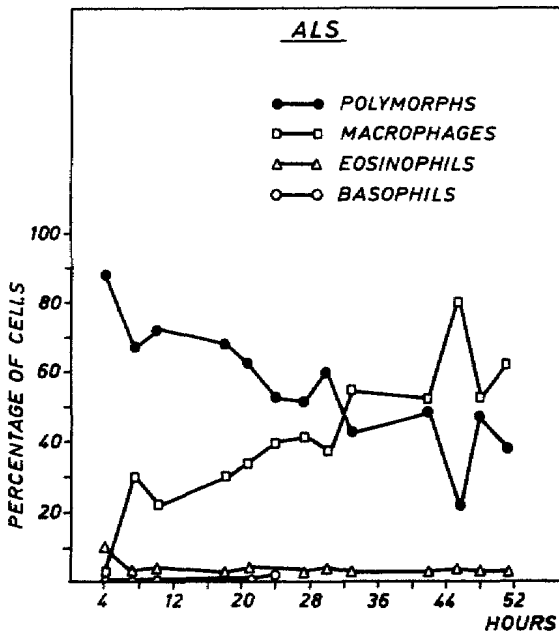


Fig. 2.

Graphic presentation of the cellular sequence in a characteristic experiment following the administration of 1 mg ALS (45 I B serum). Eosinophilic leucocytes can be seen particularly during the first 24 hours.

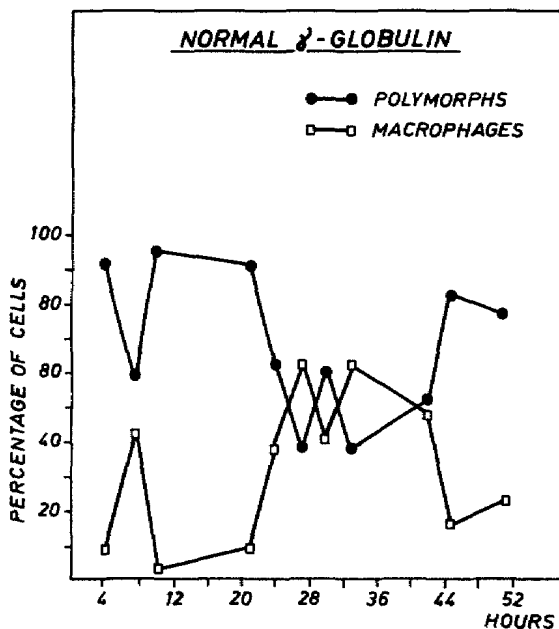


Fig. 3.

Simultaneous control experiment with administration of 1 mg normal rabbit gammaglobulin.

RESULTS

In all cases, after local administration of ALS, the skin windows showed the well-known sequence of polymorpho-nuclear leucocytes (PMN) and macrophages found in previous studies (*Riis* 1959; *Wolf-Jürgensen* 1966). After three hours there were generally more than 90 per cent PMN, but during subsequent hours the number of macrophages increased, to become more than 50 per cent after 12–15 hours. Various changes in the relationship between the two cell types could be seen, but, again, towards the end of the second day the PMN generally predominated.

There was, however, a characteristic difference between the cellular exudate after the application of ALS, and the normal cellular exudate in that the former contained many eosinophilic leucocytes, particularly during the first 24 hours. Fig. 1 is a graphic presentation of the maximum eosinophil percentage. Furthermore, there was a marked difference in the ability of the various sera to provoke eosinophilia. Sera 45 I A and 45 I B had a considerable eosinotactic effect, while serum 45 II did not differ from the simultaneous control test with normal rabbit gammaglobulin.

Out of 16 experiments with serum 45 I A, only two showed no eosinophils and half of them had 4 per cent or more. The maximum eosinophil percentage was 11 per cent. In one control experiment with normal gammaglobulin there were 4 per cent eosinophils.

Out of 11 experiments with serum I B, five had considerable eosinophilia, one with 64 per cent eosinophiles.

The number of eosinophilic leucocytes was largest during the first 24 hours and decreased during the second day.

Fig. 2 shows a characteristic curve after 1 mg ALS 45 I B serum, and Fig. 3 the simultaneous control experiment with 1 mg normal rabbit gammaglobulin. Basophilic leucocytes were rarely seen. There was no relationship between the degree of eosinophilia and the cytotoxic or agglutination titres.

DISCUSSION

This skin window study showed that ALS had no influence locally on the sequence of polymorphonuclear leucocytes and macrophages in the cellular exudate. This corresponded to what has been observed previously in unspecific inflammation in skin windows (*Riis* 1959). Thus, ALS seems not to inhibit the migration of mononuclear cells from the peripheral blood, or the transformation to "macrophages".

However, with two sera considerable eosinophilia was observed in the cellular exudate after local administration of ALS. Normally, eosinophilic leucocytes are not seen in un-

specific inflammations in skin windows (*Riis* 1959; *Wolf-Jürgensen* 1962), e.g. after application of physiological saline. Slight eosinophilia occurred, however, when 1 mg of normal rabbit gammaglobulin was applied. The reason for the considerable eosinophilia after application of ALS is not clear.

After local administration of histamine and histamine liberator (*Wolf-Jürgensen & Zachariae* 1965), eosinophilia could be produced experimentally, and also after bradykinin and serotonin (*Wolf-Jürgensen et al.* 1969 a, b). A decrease in the histamine content in the skin after universal administration of ALS has been observed (*Wolf-Jürgensen & Zachariae* 1970). The immune exudate of immediate type also contains eosinophilic leucocytes during the first 24 hours (*Hu et al.* 1961), while with delayed immunoreactions basophilia and eosinophilia are seen during the second day (*Wolf-Jürgensen* 1960).

Thus, it is possible that the action of ALS serum is a liberation of mediators of inflammatory reactions, or that the eosinophilia is an indication that an immunization process against the foreign protein is starting.

However, further investigation is necessary to determine whether there is any relationship between the degree of eosinophilia and the immunosuppressive effect of ALS.

SUMMARY

A report is given of a study of the local influence of ALS on the cellular exudate in 33 skin window tests. No effect was found on the lymphocyte migration or the transformation to macrophages. However, with two sera there was considerable eosinophilia in the cellular exudate. The significance of this finding is discussed. Further investigation may reveal whether eosinophilia has any relationship to the immunosuppressive effect of ALS.

REFERENCES

- Bach, J. F. & Antoine, B.: *In vitro* detection of immuno-suppressive activity of anti-lymphocyte sera. *Nature* 217, 658-659, 1968.
- Greaves, M. F., Tursi, A., Playfair, J. H. L., Torrigiani, G., Zanur, R. & Roitt, I. M.: Immunosuppressive potency and *in vitro* activity of antilymphocyte globulin. *Lancet* 1, 68-72, 1969.
- Hu, F., Fosnaugh, R. P., Bryan, H. G. & Jacks, D.: Human skin window. *J. invest. Derm.* 37, 409, 1961.
- Jooste, S. V., Lance, E. M., Levey, R. H., Medawar, P. B., Ruskiewicz, S. R. & Taub, R. N.: Notes on the preparation and assay of anti-lymphocytic serum for use in mice. *Immunology* 15, 697-705, 1968.
- Levey, R. H. & Medawar, P. B.: Some experiments on the action of anti-lymphoid antisera. *Ann. N. Y. Acad. Sci.* 120, 164, 1966.
- Levey, R. H. & Medawar, P. B.: The mode of action of antilymphocyte serum. *Ciba Found. Symp.* 29, 70, 1967.
- Monaco, A. P., Wood, M. L., van der Werf, B. A. & Russell, P. S.: Effects of antilymphocyte serum in mice, dogs and man. Ciba Foundation Study Group, No. 29: Antilymphocytic Serum (Ed. G. E. W. Wolstenholme & M. O'Connor), p. 111. Churchill, London 1967.
- Rebuck, J. W.: On the role of the monocyte in inflammation as demonstrated by a new technique. *Anat. Rec.* 76, 46, 1940.
- Riis, P.: The cytology of inflammatory exudate. Thesis, Munksgaard, Copenhagen 1959, p. 45-100.
- Wolf-Jürgensen, P.: Basophilic leukocytes in delayed hypersensitivity. Experimental studies in man using the skin window technique. Thesis, Munksgaard, Copenhagen 1966.
- Wolf-Jürgensen, P.: Cytological examination of experimental contact allergy using the skin window technique. *Acta allerg. (Kbh.)* 17, 574, 1962.
- Wolf-Jürgensen, P., Juel Henningsen, S. & Zachariae, H.: Bradykinin induced eosinophilia in "skin windows". *Acta allerg. (Kbh.)* 24, 157, 1969.
- Wolf-Jürgensen, P. & Juel Henningsen, S.: Influence of serotonin (5-hydroxytryptamine) on the cellular exudate in skin windows. *Acta allergol. (Kbh.)* 25, 48, 1970.
- Wolf-Jürgensen, P. & Zachariae, H.: Influence of antilymphocyte serum on skin histamine in mice. *J. invest. Derm.* 54, 178, 1970.
- Woodruff, M. F. A., Panus, K., Andersen, N. F. & Reid, B. L.: *In vivo* and *in vitro* properties of antilymphocytic serum. *Ciba Found Symp.* 29, 57, 1967.

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