

From Medical Department F (Head: Michael Schwartz, M.D.),
The Glostrup Hospital, Copenhagen, Denmark.

BASOPHILIC LEUKOCYTES IN EXPERIMENTAL TUBERCULIN REACTIONS STUDIED BY THE SKIN-WINDOW TECHNIQUE¹

By

PER WOLF-JÜRGENSEN

INTRODUCTION

The tuberculin reaction is the traditional model of cellular or delayed allergic reaction. Previous histological studies of biopsies have shown that the reaction is characterized by infiltration by mononuclear cells in the epidermis and corium, affecting especially the perivascular areas (*Crowle* 1962, *Waksman* 1960). The tuberculin reaction has previously been studied by the skin-window technique by *Rebuck & Yates* (1954) who found a delayed appearance of mononuclear cells in the cellular exudate. In previous experiments employing the skin-window technique in experimental contact allergy induced by dinitrochlorobenzene (DNCB)—as the prototype of a cellular or delayed reaction—basophilic leukocytes have been a characteristic finding in the exudate (*Wolf-Jürgensen* 1962). It seemed natural, therefore, to study the tuberculin reaction as well. The results of such investigations will be reported below.

MATERIAL AND METHODS

A total of 17 patients was studied, 12 males in the age range 18-54 years and 5 females aged 23-57 years. Most of the patients were suffering from minor surgical diseases. One had previously been treated for tuberculosis. 11 tuberculin-positive and 2 tuberculin-negative patients were examined by the skin-window

¹ Aided by a grant from the Danish Science Foundation.

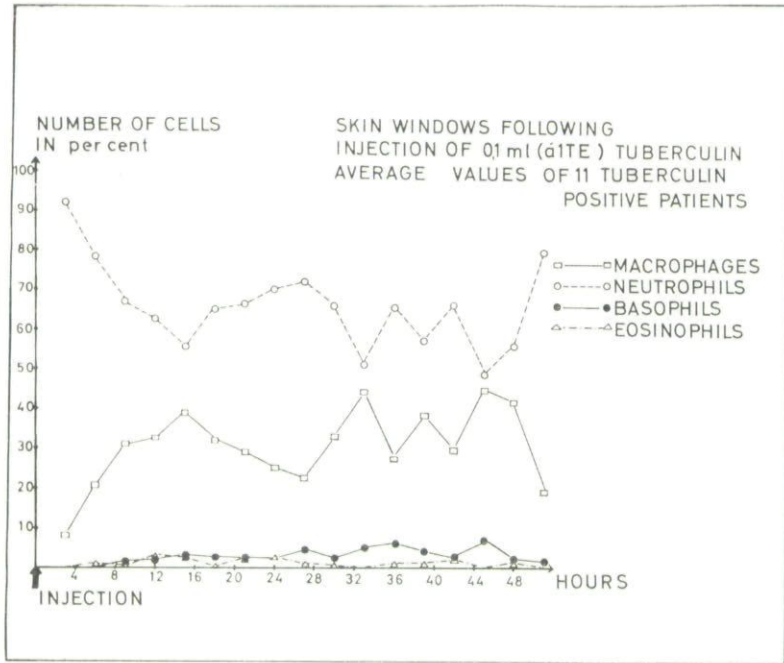


Fig. 1.

Graphic representation of the sequence of cells in the exudate in 11 tuberculin-positive patients following injection of 1 T.U. tuberculin.

Note the basophilic leukocytes.

technique. This was done by purified tuberculin (1 T.U./0.1 ml), in the case of tuberculin-negative subjects by 10 T.U./0.1 ml). Two experiments were performed on each patient, tuberculin being applied to one arm and physiological saline to the other. Moreover, all the patients had intracutaneous tuberculin tests in which the reaction was read at the end of 72 hours.

The skin-window investigation was performed by the technique of *Rebuck* (1940). The epithelium in an area of approximately 1 cm² on the volar surface of the forearm was scraped off with a sterile knife, and 0.1 ml tuberculin—in the control experiments physiological saline—injected intradermally with a Mantoux needle. Thereupon, the area was covered with the first of a series of sterile cover slips fortified with a piece of cardboard, and fixed with adhesive tape. The cover slips were changed every 3 hours through 51 hours, stained by the May-Grünwald-Giemsa method, (*Riis*, 1959) mounted on slides, and differential counts were performed. At least 200 cells were counted. A total of about 500 preparations were made.

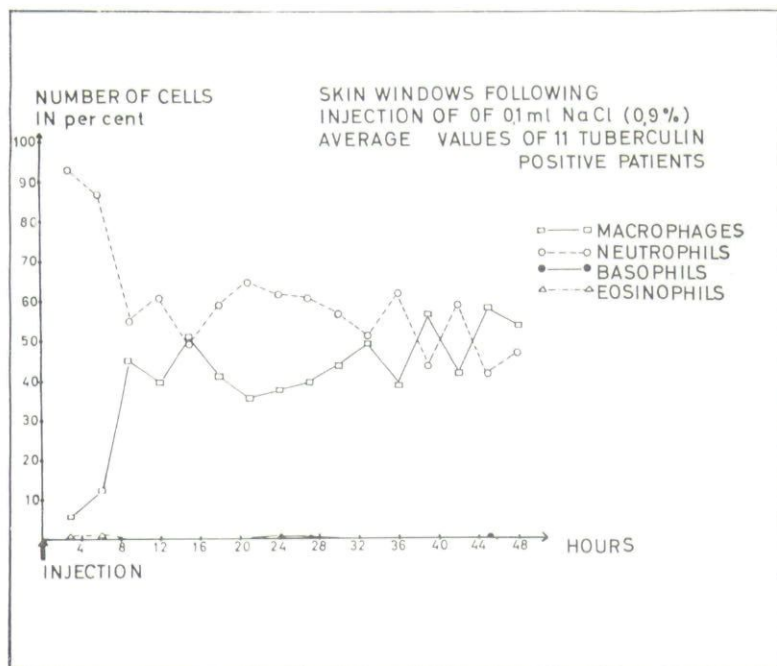


Fig. 2.

The cellular sequence in the same 11 patients in concurrent control experiments using 0.9 per cent NaCl.

RESULTS

The gross reactions were characterized by induration which, at the end of 72 hours, measured 6–16 mm in diameter, surrounded by erythema. The negative reactions measured 0–2 mm, but in several cases erythema was present.

Differential counts showed in all cases the well-known sequence regarding polymorphonuclear leukocytes and macrophages (Riis 1959). On the other hand, the 11 tuberculin-positive individuals showed constantly basophilic leukocytes, ranging from a maximum of 2 to 15 per cent in the exudate, especially on the 2nd day; eosinophilic leukocytes were also present in a number of cases, but less constantly. The number of basophils was at a maximum in the patients who showed

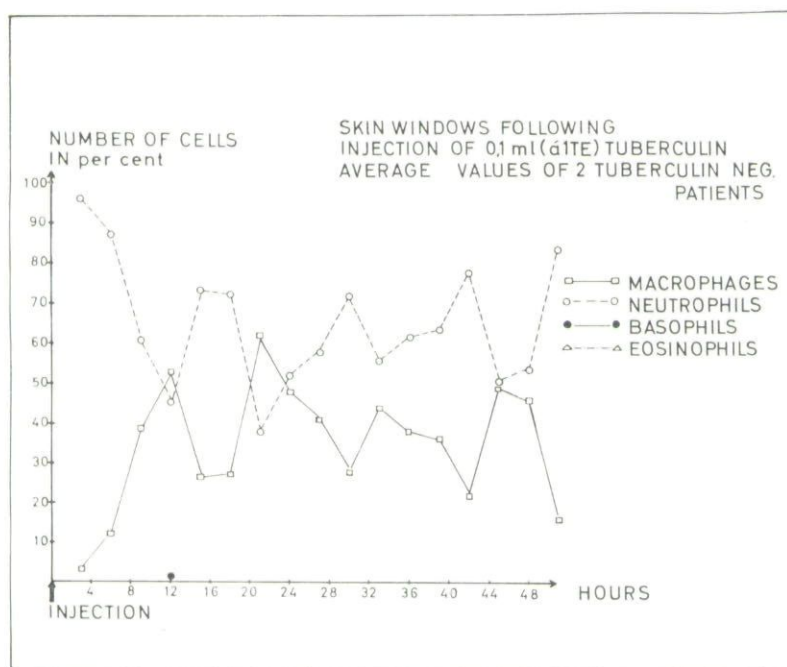


Fig. 3.

The cellular composition of the exudate in two tuberculin-negative patients following injection of 1 T.U. tuberculin.

the most marked gross reactions. No basophils, or only a few, were observed in the concurrent control experiments using physiological saline or in the experiments on tuberculin-negative subjects.

Fig. 1 shows the sequence of the cells in the exudate, set out graphically for 11 tuberculin-positive patients. Four types of cell were observed: polymorphonuclear leukocytes, macrophages, eosinophilic and basophilic leukocytes. At the end of 3-4 hours, there were eosinophils, and at the end of 8 hours basophils. Especially at the end of 30 hours, there was an accumulation of basophilic leukocytes, a reaction which was at its peak at the end of 45 hours. Thereafter, degranulation of the cells was often observed. The eosinophils were less constant and less numerous, the greatest number being seen in the

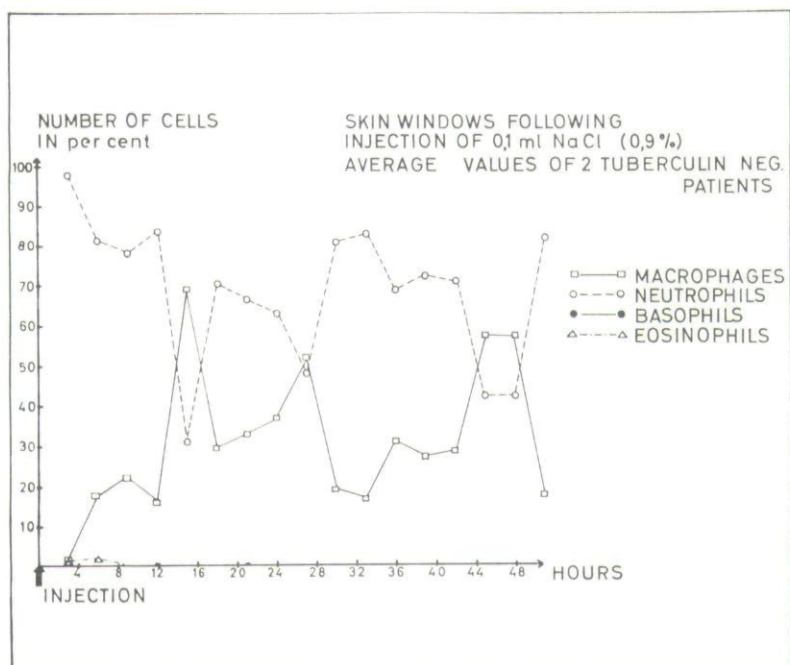


Fig. 4.

Concurrent control experiments on the same two patients,
following injection of 0.1 ml 0.9 per cent NaCl.

first 24 hours. At the outset, the polymorphonuclear cells were predominant, but from the 3rd hour the number of macrophages decreased. In many of the experiments a change occurred between the 8th and 16th hour after which macrophages predominated. Thereupon, the number of polymorphonuclears again increased, but another increase of macrophages was observed in 30 hours, until the polymorphonuclears predominated towards the end of the experiment.

Fig. 2 shows the cellular sequence in the exudate in the same 11 patients in the concurrent experiments using physiological saline. Very few preparations showed occasional basophils, but not more than 1.5 per cent.

Figs. 3 and 4 show the cellular composition of the exudate in two tuberculin-negative patients after injection of tuberculin

and of physiological saline respectively. There were practically no basophilic or eosinophilic leukocytes, while the sequence of polymorphonuclear leukocytes and macrophages was the same as in the tuberculin-positive individuals.

DISCUSSION

The investigation showed that basophilic leukocytes constitute a constant finding in the exudate in the tuberculin reaction. On the other hand, they do not occur in concurrent control experiments using physiological saline. The experiments revealed, moreover, that the tuberculin antigen itself does not give rise to the appearance of basophils in the exudate, as none was found after the injection of tuberculin into tuberculin-negative individuals. This is in keeping with the results of previous experiments (*Wolf-Jürgensen* 1962) on experimental contact allergy to DNCB in which DNCB failed to induce the appearance of basophils in non-sensitized persons.

Accordingly, the basophilic leukocytes appear to be characteristic of antigen-antibody reactions of the delayed type. In other experiments basophils have been demonstrated in the exudate following application of diphtheria toxin to patients who had been vaccinated against diphtheria (*Wolf-Jürgensen, Anthonisen & Riis* 1964) and following transplantation of lymphocytes (*Wolf-Jürgensen & Schwartz* 1964)—the so-called NLT test (*Brent & Medawar* 1963) which must also be assumed to be of the delayed type.

The finding of basophilic leukocytes in delayed allergic reactions has been confirmed by others (*Juhlin* 1963, *Aspegren, Fregert & Rorsman* 1963 a, 1963 b). The latter authors also studied the tuberculin reaction, but they found an increased number of basophilic leukocytes only in about half the cases in cantharidin bullae applied to the gross tuberculin reaction (1964).

It may seem peculiar that basophilic leukocytes have not been described previously in allergic reactions of the delayed type. Histological investigations, especially of biopsy spec-

imens, have shown infiltration with mononuclear cells in the epidermis and corium, especially in the perivascular areas, and intracellular oedema (*Crowle* 1962, *Gell* 1961, *Waksman* 1960).

Braunsteiner, Paertan & Thumb (1958) and *Nexmand* (1954), using the cantharidin bullae technique, did not find basophilic leukocytes in the tuberculin reaction. Biopsies, and to some extent also investigations by the cantharidin bullae technique, give only a "snapshot" of the reaction, perhaps at a time when very few basophils are present in the exudate. By the skin-window method it is possible to study the exudate from the same reaction many times. However, *Rebuck & Yates* (1954), studying the tuberculin reaction by the skin-window technique by dropping 0.5 ml of Koch's Old Tuberculin into the skin window, found no basophils. In non-specific inflammations basophilic leukocytes are not seen (*Riis* 1959).

Morphologically, the cells are exactly similar to basophilic cells in the blood. The nucleus is lobulated, the granules coarse, and the shape of the cell regular, whereby it may be distinguished from connective-tissue mast cells. Immature forms are not seen. It must be assumed, therefore, that the cells have migrated from the blood. The relative number of basophilic leukocytes in the allergic exudate may be more than 50 times that in the blood, while these cells do not occur in concurrent control experiments using physiological saline. Thus, the antigen-antibody reaction appears to make an increased number of basophils migrate from the vessels at the site of the reaction and/or bind those basophils which pass from the blood at the site of the reaction. The amount reaches a maximum at the end of 36-48 hours at which juncture degranulation appears in the preparations.

Since basophilic leukocytes are not observed in non-specific inflammations, investigations by the skin-window technique may be used in deciding whether a positive tuberculin reaction is specific.

SUMMARY

Basophilic leukocytes are a constant finding in the exudate from experimental tuberculin reactions studied by the skin-window technique. On the other hand, they are not present in concurrent control experiments using physiological saline or in the exudate in tuberculin-negative individuals. Investigation by the skin-window technique may be of value in distinguishing between a positive tuberculin reaction and a non-specific reaction.

REFERENCES

- Aspegren, N., Freger, S. & Rorsman, H.*: Basophil leukocytes in allergic patch test reactions. *J. Invest. Derm.* 41, 107 (a), 1963.
- Aspegren, N., Freger, S. & Rorsman, H.*: Basophil leukocytes in allergic eczematous contact dermatitis. *Int. Arch. Allergy* 23, 150 (b), 1963.
- Aspegren, N., Freger, S. & Rorsman, H.*: Basophil Leukocytes at Sites of Intracutaneous Tuberculin Test Reactions. *Int. Arch. Allergy* 24, 35, 1964.
- Braunsteiner, H., Paertan, J. & Thumb, N.*: Studies on lymphocytic function. *Blood* 12, 417, 1958.
- Brent, L. & Medawar, P. B.*: A new approach to the "typing" problem. *Brit. med. J.* II, 269, 1963.
- Crowle, A. J.*: Delayed Hypersensitivity in Health and Disease. pp. 26-28. Charles C. Thomas. Illinois. 1962.
- Gell, P. C. H.*: In Cellular and Humoral Aspects of Hypersensitive States. H. S. Lawrence, ed. New York. Poul B. Hoeber. pp. 43-66. 1959.
- Juhlin, L.*: Basophil leukocytes in blood and inflammatory exudate. *Acta Dermat.-Venereol.* 43, 528, 1963.
- Nexmand, P. H.*: Cytologic studies of allergic reactions by means of cantharides blisters. *Acta Dermat.-Venereol.* 34, 389, 1954.
- Rebuck, J. W.*: On the rôle of the monocyte in inflammation as demonstrated by a new technique. *Anat. Rec.* 76, 46, 1940 a.
- Rebuck, J. W.*: Demonstration of the monocyte in inflammation as shown by a new technique. *Anat. Rec.* 76, 93, 1940 b.
- Rebuck, J. W. & Yates, J. L.*: The cytology of tuberculin reactions in skin-windows in man. *Amer. Rev. Tuberc.* 60, 216, 1954.
- Riis, P.*: Cytology of Inflammatory Exudate. Thesis. Copenhagen. 1959.
- Waksman, B. H.*: In Cellular Aspects of Immunity. G. E. W. Wolstenholme & M. O. Connor, eds. Boston, Little, Brown & Co., pp. 280-329, 1960.
- Wolf-Jürgensen, P.*: Cytological examination of experimental contact allergy using the skin-window technique. *Acta Allergol.* 17, 547, 1962.

- Wolf-Jürgensen, P., Anthonisen, P. & Riis, P.:* The Cutaneous Abacterial Inflammatory Reaction in Patients with Ulcerative Colitis. 1964. In press.
- Wolf-Jürgensen, P. & Schwartz, M.:* Normal-lymphocyte transfer in man. Basophil leucocytes in delayed skin reaction. *Lancet II*, 388, 1964.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.