

LYMPH-NODE PERMEABILITY FACTOR: A MEDIATOR IN DELAYED HYPERSENSITIVITY?

Experiments using the Skin Window Technique

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Abstract. The cellular exudate following intradermal injection of LNPF was studied in 12 persons by the skin-window technique. The cellular sequence differed from that in simultaneous control tests using saline in containing basophilic and eosinophilic leukocytes, especially on the second day after the injection, as seen in reactions of the delayed hypersensitivity type. Extracts from Tb-sensitized as well as from normal lymph nodes were effective, although the latter seemed to give weaker reactions. The results indicate that LNPF is a mediator in delayed hypersensitivity.

Extracts from normal and sensitized lymph nodes contain a principle which increases vascular permeability (1) and produces diapedesis of leukocytes (4) as well as deposition of a fibrinoid material (6, 7, 8). This factor, called the lymph-node permeability factor (LNPF), has also been found to accumulate in the tuberculin reaction and in 2, 4 DNCB contact dermatitis in guinea-pigs (10), and may be distinguished from other known permeability factors (3). Its chemical structure is unknown. LNPF has been isolated from a number of animal species, including man, and seems not to be species-specific. In vitro experiments have shown that LNPF is released from lymphoid cells upon contact with an antigen (9). Schild & Willoughby (3, 5) have advanced the hypothesis that LNPF is a mediator in delayed hypersensitivity. Moreover, it has been demonstrated by Casciani & Cortesini (1969) that LNPF inhibits renal allografts in rats (1).

MATERIAL AND METHOD

The experiment comprised a total of 12 human subjects. In four, the LNPF from Tb-sensitized and normal guinea-

pigs respectively was studied in simultaneous tests on both arms. On 4 subjects the LNPF from normal guinea-pig lymph nodes was studied with saline control tests. Lastly, on 4 persons LNPF from Tb-sensitized lymph nodes was studied with saline control tests. No subject had a family history of allergy or haematological disease.

The skin-window study was carried through as described previously (2, 12): After scraping off the epidermis on the volar aspect of the forearm, the area was injected intracutaneously with 0.1 ml extract and saline respectively. Thereafter, the cover slip was applied and changed every 3 hours through 48 hours, stained by the method of May-Grünwald-Giemsa, examined in the microscope, and differentially counted.

For preparing the LNPF, the technique of Schild and Willoughby (3) was used. Lymph nodes of normal as well as of Tb-sensitized guinea-pigs were extracted. The guinea-pigs were sensitized by subcutaneous injections of 4 × 0.1 ml of a suspension of killed tubercle cells and skin-tested at the end of 3 weeks. The removed lymph nodes were crushed in Hanks solution. The suspension, containing approximately 250 × 10⁶ lymphocytes/ml, was exposed to alternate freezing and thawing, and thereafter centrifuged, 4,000 rpm, for 10 min. Finally, the cell-free supernatant was filtered through sterile bacteriological filters.

RESULTS

The investigations showed that the cellular sequence following injection of LNPF differs in a characteristic manner from the simultaneous control test in containing eosinophilic and basophilic leukocytes. The highest counts were seen on the second day.

Figs. 1, 2 and 3 give the average values representing the sequence of basophilic and eosinophilic leukocytes in eight experiments using extracts from Tb-sensitized lymph nodes, normal

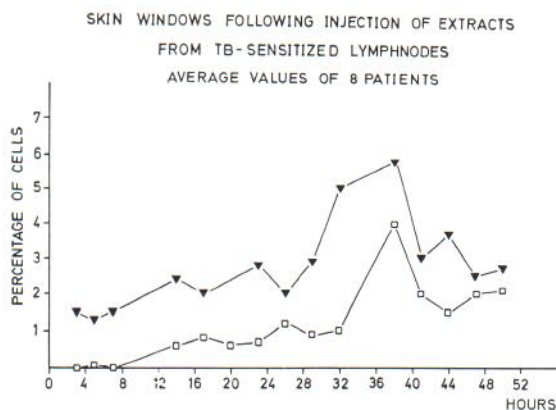


Fig. 1. The sequence of basophils and eosinophils in the inflammatory exudate following application of extracts from Tb-sensitized lymph nodes. ▼—▼, eosinophils; □—□, basophils.

lymph nodes, and in control tests using saline, respectively. The largest numbers of basophils and eosinophils in one experiment were seen following injection of LNPF from Tb-sensitized lymph nodes. The values in the experiments ranged between 14% eosinophils and 12% basophils, and 2% and 1% respectively. The cells may be observed already at the end of some hours, but are most numerous on the second day, at about 36 hours; thereafter, they decrease in number.

After injection of LNPF from normal lymph nodes the curve was of a similar course, but the eosinophilia and basophilia were not so marked in these experiments (ranging between 7% and 2% eosinophils and 6% and 0% basophils). The

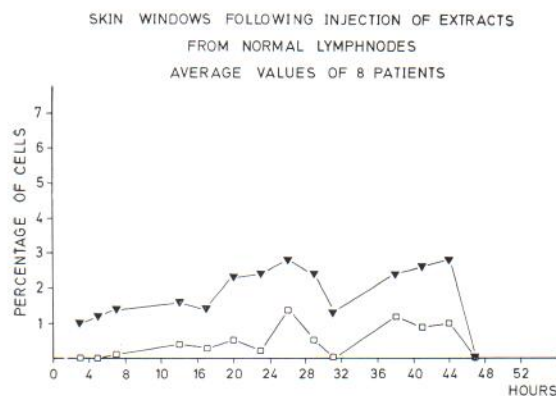


Fig. 2. The occurrence of basophils and eosinophils following application of extracts from normal lymph nodes. ▼—▼, eosinophils; □—□ basophils.

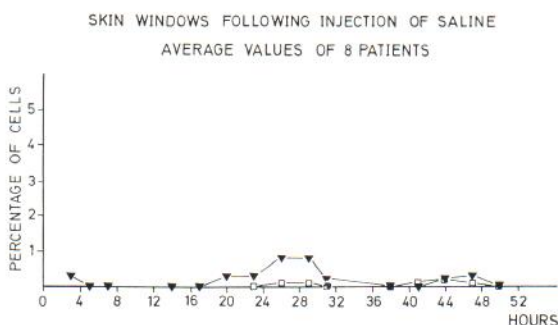


Fig. 3. The cellular sequence in simultaneous control experiments using saline. ▼—▼, eosinophils; □—□, basophils.

simultaneous control tests using saline seldom showed eosinophilic and basophilic cells.

In all 12 tests, differential counts showed the well-known sequence of polymorphonuclear and mononuclear leukocytes (10). Figs. 4 and 5 contrast LNPF-injected with sensitized lymph nodes, and the simultaneous control with saline. The polymorphonuclears predominated at the outset, but the number of mononuclears increased and made up the greater part after the lapse of 9 hours. Later, towards the end of the period, there was again a preponderance of polymorphonuclears.

DISCUSSION

Previous investigations using the skin-window technique have shown that basophilic and eosino-

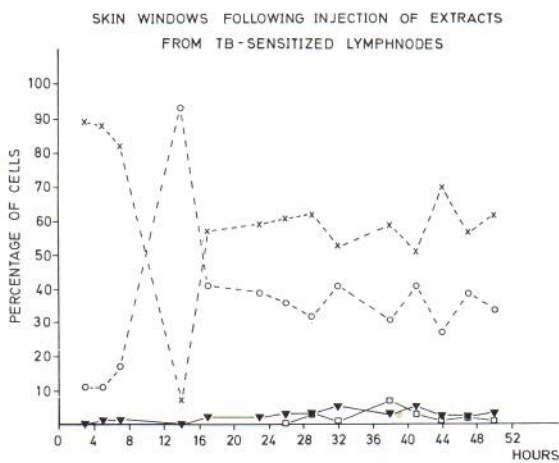


Fig. 4. Characteristic cellular sequence in one person following injection of extract from Tb-sensitized lymph nodes. ×---×, polymorphs; ○---○, macrophages; ▼—▼, eosinophils; □—□, basophils.

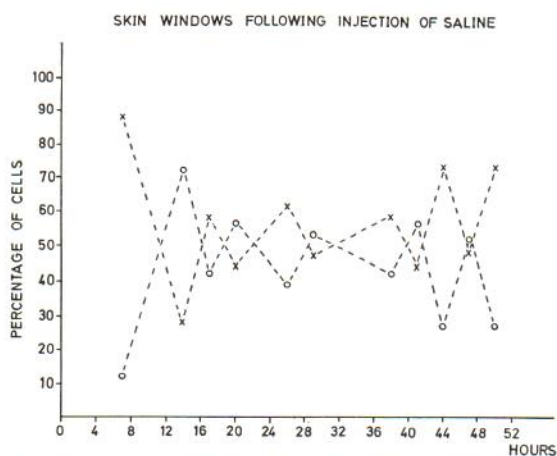


Fig. 5. Simultaneous control experiment in one person using saline. x---x, polymorphs; o---o, macrophages.

philic leukocytes are characteristic of cellular exudates of the delayed type, while these cells are not present in non-specific inflammations (11).

Other mediators of inflammatory processes have previously been investigated by the skin-window technique. For instance, it has been demonstrated that histamine and the histamine liberator polymyxin B give rise to the appearance of eosinophilic leukocytes, especially during the first hours after the injection (14), as does bradykinin, but no basophils were seen in these experiments (13). Accordingly, extracts from lymph nodes appear to contain a factor which influences the cellular sequence in a way that is different from that caused by the other mediators, the sequence resembling that found in experimental reactions of the delayed hypersensitivity type.

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