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Density of Langerhans' Cells in ATPase Stained Epidermal Sheet Preparations from Stasis Dermatitis Skin of the Lower Leg

FRIEDRICH A. BAHMER and HEIKE LESCH

Department of Dermatology, University Hospital Homburg (Saar), FRG

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The density of Langerhans' cells (LC) stained with ATPase was determined in epidermal sheet preparations in skin specimens from patients with stasis dermatitis of the lower leg. Their density was highest in areas adjacent to manifest stasis dermatitis areas. Clinically diseased skin contained less LC than the adjacent areas, but still more than skin from control patients without venous insufficiency. The increased number of ATPase positive cells within and around stasis dermatitis skin might contribute to the high number of contact allergies observed in this patient group because of the antigen presenting capacity of these cells. *Key words: Leg ulcers; Contact dermatitis; ATPase staining.* (Received September 16, 1986.)

F. A. Bahmer, Universitäts-Hautklinik, D-6650 Homburg (Saar), West Germany.

Chronic venous insufficiency (CVI) and its sequelae such as stasis dermatitis and leg ulcer, rather frequent disorders, are very often associated with allergic sensitizations to topically applied medicaments and their vehicles (1).

Virtually nothing is known of systemic or local factors influencing the induction and persistence of contact allergies in this patient group.

In the skin, antigen presenting cells such as Langerhans' cells (LC) are involved in the eliciting phase of type IV allergic sensitization (2). Therefore, the present study was performed to determine the density of LC in stasis dermatitis skin from the lower leg with the adenosine triphosphatase (ATPase) method (3). Additionally, the number and appearance of the dendrites of the individual LC was determined.

MATERIAL

Punch biopsies from the dorsal aspect of the calf were obtained from 32 patients with pronounced stasis dermatitis, clinically characterized by induration and pigmentary changes such as dermite ocre (4). In all cases, the stasis dermatitis was the result of long standing CVI. The biopsy sites were classified into three groups: group I a consisted of 5 men and 5 women between 29 and 82 years (mean 52.3) with clinically pronounced stasis dermatitis. In this group, the biopsies were taken from clinically uninvolved skin adjacent to the stasis dermatitis area within a distance of about 5 (±3) cm.

Group I b consisted of 12 patients, 6 men and 6 women between 19 and 82 years (mean 60.4) with the same degree of stasis dermatitis as in group I. In this group, however, the biopsies were taken from the stasis dermatitis area itself.

Group II served as control group. It consisted of 6 men and 4 women between 31 and 79 years (mean 60.7) without any sign of stasis dermatitis or CVI which were hospitalized for other skin diseases neither involving the skin of the lower leg nor the vascular system.

METHOD

After informed consent had been obtained, 6 mm punch biopsies were taken under local anaesthesia, in all cases from the dorsal aspect of the calf.

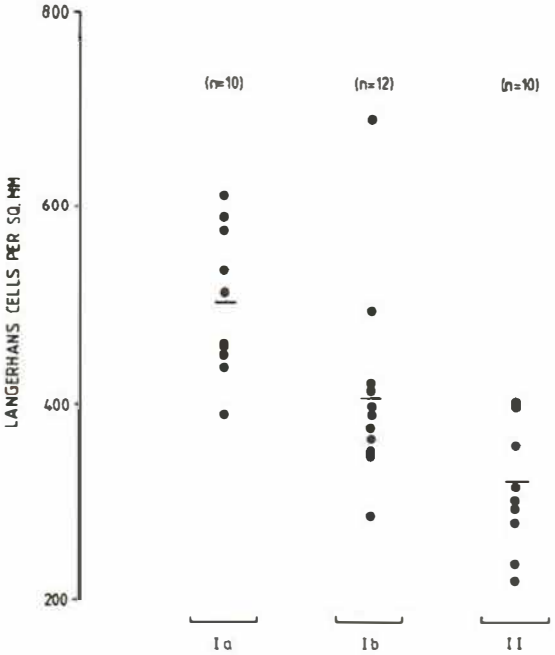


Fig. 1. Density of ATPase stained LC in stasis dermatitis of the lower leg. Ia: clinically uninvolved skin, adjacent to stasis dermatitis area. Ib: clinically involved skin. II: control group without stasis dermatitis or CVI (differences between groups $p<0.001$).

Immediately after the biopsy was taken, epidermal sheet preparations were processed according to the method of Juhlin & Shelley (3). Briefly, the epidermis was separated from the dermis after incubation in phosphate buffered EDTA for 2 h at 37°C. The sheets were then fixed in cacodylate formaldehyde solution for 20 min at 4°C. After washing and incubation in ATP-Pb solution for 60 min at 37°C, staining of the LC was completed with immersion into ammonium sulfide solution for 20 min at room temperature. After mounting in glycerol jelly the specimens were stored in the dark at 4°C until assessment, which was performed within 24 h.

The density of the cells and their morphology was evaluated with a Zeiss Photomicroscope II at a magnification of 160-fold. To avoid bias by only choosing evenly looking areas, twenty fields were selected at random in each specimen. In addition to the number of LC per square millimeter, the number of dendrites per LC was counted. Only cell processes with clearly visible origin at the cytoplasm and with a length of at least the diameter of the cytoplasm itself were regarded as dendrites.

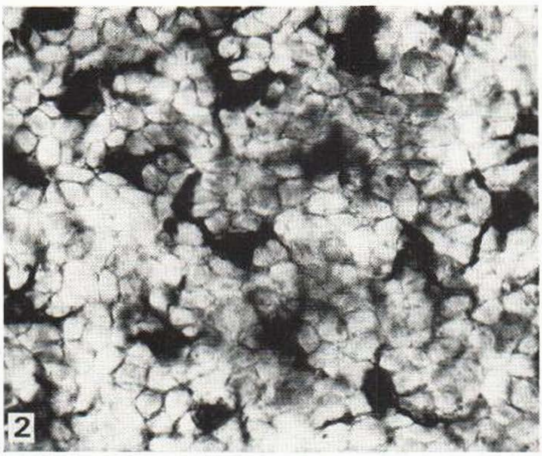
The figures obtained were compared with the analysis of variance. The calculation was done with the ANOVA-program on a Hewlett Packard desktop computer.

RESULTS

The number of LC in the different groups is shown graphically in Fig. 1. Skin from areas adjacent to clinically altered skin (group I a) showed the highest density of LC with 388 to 626 LC per sq. mm (mean 509.3). In stasis dermatitis skin itself (group I b), the mean density of LC was lower than in group I a skin (275 to 713 LC per sq. mm, mean 406.8). The epidermis of control patients (group II) showed a lower density of LC than both group I a and group I b. Analysis of variance showed highly significant ($p<0.001$) differences between the three groups.

Generally, the LC in preparations from the control group II appeared better delineated with rather sharply defined dendrites (Fig. 2) than those from both group I a and group I b. In the latter, the LC appeared plump and with shorter and less well defined dendrites (Fig. 3).

The number of dendrites varied between and in all groups. Statistical analysis did not disclose significant differences between the groups.



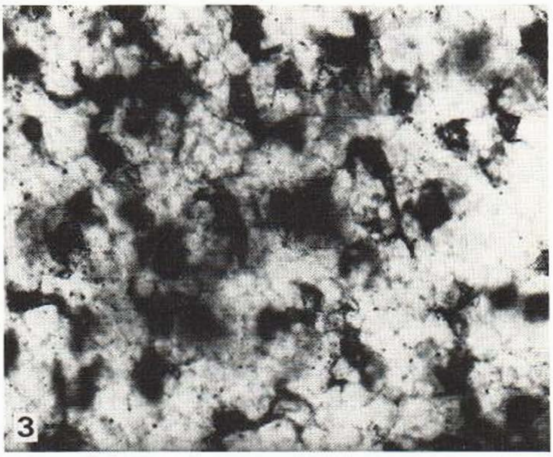


Fig. 2. ATPase stained LC in normal skin from the lower leg. Sharply defined cells with long, branching dendrites (×105).

Fig. 3. ATPase stained LC from stasis dermatitis skin from the lower leg. Less well defined cells with fewer and shorter dendrites (×105).

DISCUSSION

It is a commonplace that dermatologic patients with stasis dermatitis or leg ulcers are prone to develop contact allergies not only to topically applied medicaments, but also to their vehicles. Furthermore, the average number of sensitization to different chemicals is about twice than that in patients with other skin diseases. Data collected by Dooms-Goossens on more than 6000 sensitized individuals show that in this patient group, in addition to the higher sensitization rate, the average age in which contact allergies occur is much higher (personal communication).

Usually, impairment of the barrier function of the skin and the presence of mononuclear cells are considered as promoting factors for the development of contact allergies in patients with stasis dermatitis (5).

Epidermal LC play an important role for the induction and persistence of contact allergies, together with other immunocompetent cells (6). It is reasonable to assume that they are involved in the process of sensitization in these cases. However, epidermal LC are not an absolute prerequisite, since sensitization may easily be induced by the application of known contact sensitizers such as benzoyl peroxide to leg ulcers devoid of epidermis (7). Here too, the high number of HLA-DR positive cells within the dermis might act as antigen presenting cells (8).

Mapping of LC in skin specimens from different body areas and with different methods did not disclose differences concerning anatomical site or sex independent from the method of demonstration (9, 10). However, with histochemical and immunohistochemical methods differences in the absolute number of LC per area unit are obtained (11).

The present study shows that the epidermis from stasis dermatitis skin of the lower leg contains a higher number of LC than normal skin from the same site. This finding could be confirmed in a small number of patients with unilateral stasis dermatitis in whom biopsies from both the involved and uninvolved leg could be investigated. Here too, a significant difference in LC density was found (unpublished observations).

Interestingly, the number of LC is even higher in normal appearing skin adjacent to clinically diseased skin than in stasis dermatitis skin itself. In contrast to acute contact

dermatitis reactions, in which the number of LC is markedly decreased (11), stasis dermatitis skin shows an increased number of LC. One possible explanation might be that in these areas a mild irritant reaction augments the number of LC, or that the process of their replacement is impaired (12). Although the density of LC differs significantly in the areas investigated, no such difference was found for the number of dendrites. This might be due to the fact that assessment of dendrites is highly subjective and might further be biased by the rather high variability in the thickness of the epidermal sheet preparation, reflecting the irregular acanthosis often seen in stasis dermatitis.

Apart from other factors such as a possible reservoir function of the epidermis for contact allergens (13) and the presence of large numbers of HLA-DR positive cells in the dermis of stasis dermatitis skin (8), the increased number of LC demonstrated might well contribute to the induction of contact allergies so frequent in these patients.

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