Acta derm.-venereol. 47: 150–153, 1967

SUCTION BLISTERS IN THE STUDY OF CELLULAR DYNAMICS OF INFLAMMATION

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Several methods have been developed for the study of cellular dynamics in cutaneous inflammation. Besides conventional biopsy specimens (19), different cytological sampling methods have been employed. The acantholytic cantharidine blister lends itself to the study of basophils in delayed hypersensitivity reactions of the tuberculin or contact type (1, 3) in spite of the fact that it attracts neutrophils and eosinophils (18).

Rebuck's skin window technique (15) has gained wide popularity in the study of immediate- (5), delayed- (21, 22), and macrophagic-types of hypersensitivity (16). Lymphocytes, however, show a poor adhesion to glass surface (4, 12). In addition, the trauma induced with a knife or sandpaper cannot be standardized regarding depth and area. However, controlled, purely epidermal abrasion may be produced with a finishing dental burr (13). In spite of the method used, there remains the inherent leukotactic effect of epidermal breakdown with an eosinophilic component (20). Furthermore, the glass surface of the skin window coverslips activates the Hageman factor, which enhances inflammation (7); the precipitated fibrin also acts as an eosinotaxin (17).

The unpredictable possibility of infection, enhanced by the repeated change of the coverslips, cannot be avoided in the skin window technique (8, 9). Fifteen pyogenic staphylococci under the coverslip may be sufficient to evoke a seropurulent exudate containing six million organisms within 24 hours (6).

Even the more quantitative modification of the skin window technique the glass chamber method of Perille and Finch (14) has several of these drawbacks.

Compared with the other methods, the suction blistering (10), which provides a sterile, non-inflammatory "chamber" between the gently separated epidermis and dermis, appears to fill the requirements of a standardized method for the study of cellular dynamics in inflammation.

Experimental

Standard suction blisters were produced using a pressure of –20 cm Hg with an improved suction device.[1] Within one to two hours of constant suction tiny vesicles appeared. Shortly thereafter the vesicles coalesced to form blisters. Suction was discontinued when the blisters had a diameter of 3–4 mm with a fluid content of 15–25 μl. Subjects with abnormal capillary resistance or with strong hair growth on the forearms were not accepted for this study in order to preserve optimally atraumatic conditions.

In the first experiment the cellular re-

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Table 1. *Number and percentage of different leukocytes in 10×7 intact blisters*

Hours	Average number of cells in blisters	Neutrophils %	Eosinophils %	Basophils %	Mononuclear cells %
0, 1, 2, 4	0	0	0	0	0
6	10	100	0	0	0
12	40	95	0	0	5
24	200	93	0	0	7

sponse evoked by suction blistering itself was studied. Sampling of the cells from intact blisters occurred immediately and 1, 2, 4, 6, 12 or 24 hours after the blister had reached its final size. At each time-point ten blisters were examined.

In the second experiment the roofs of 20 freshly formed blisters were perforated by a minute scalpel incision. Cellular emigration was followed by sampling after 1, 2, 3, 4, 6 and 12 hours.

In the third experiment a drop of diphtheria toxoid, generally used as a stimulus of inflammation, was introduced into 20 perforated blisters. Cell sampling was timed until 12 hours as above.

In the fourth experiment cellular contents were studied from vesicles, raised on ten patch test sites (nickel and balsam of Peru) 1.5 to 2.5 days from the beginning of the exposure. Since even the initial suction vesicles tended to disrupt, sampling was performed only once, i.e. one hour after their appearance.

In all experiments specimens for microscopic study were obtained and treated in the following way. From each blister two smear preparations were made with aspirated fluid. The blister roof was then removed and spread on a glass slide with the basal side up and covered with another slide. After drying the two slides were separated. Finally, imprints from denuded blister bases were made on two slides. All the air-dried slides were stained with May-Grünwald-Giemsa. When possible, all leukocytes of each slide were counted, and the sum of the six slides was considered to reflect the total number of emigrated cell population in each individual blister.

Results and Discussion

Inflammatory cells were never encountered in fresh intact blisters. Neither were they observed in 1-, 2-, nor 4-hour blisters (Table 1). After six hours occasional neutrophils were observed. Even in blisters cut at 24 hours their number was remarkably low, on an average, 200 cells per blister. Mononuclear cells were found first in 12 hour blisters, but their proportion remained insignificant. Eosinophils or basophils were never encountered within 24 hours in these 70 intact blisters.

In 20 perforated blisters the leukocyte response was slightly increased. Neutrophils appeared at four hours, and at 12 hours 1200 cells on an average could be collected (Table 2). At this time 11 per cent mononuclears, 2 per cent eosinophils, but no basophils were found.

In 20 blisters stimulated by diphtheria toxoid an accelerated inflow of inflammatory cells was noticed. Neutrophils appeared within one hour, eosinophils within four hours and basophils within twelve hours (Table 3). At this stage of inflammation the total number of emigrated cells was not countable.

Basophils were found in eight of the ten patch test sites studied. They formed 3 to 10 per cent of the leukocytes in the one-hour old suction vesicles. The proportion of neutrophils and eosinophils among the leukocytes varied widely in this small series. In two of the cases oozing of blood into the vesicles made the counting of inflammatory cells unreliable.

It is emphasized that in the intact suction blisters leukocytes did not appear until 6 hours after blister production and that

Table 2. *Number and percentage of different leukocytes in 20 perforated blisters*

Hours	Average number of cells in blisters	Neutrophils %	Eosinophils %	Basophils %	Mononuclear cells %
1–2	0	0	0	0	0
4	40	100	0	0	0
6	100	96	0	0	4
12	1200	87	2	0	11

Table 3. *Number and percentage of different leukocytes in 20 blisters stimulated by diphtheria toxoid*

Hours	Average number of cells in blisters	Neutrophils %	Eosinophils %	Basophils %	Mononuclear cells %
1	120	100	0	0	0
2	680	99	0	0	1
4	>2000	79	2	0	19
6	>2000	60	4	0	36
12	not countable	20	4	2	74

the number of these mostly neutrophilic cells remained minimal during the first 24 hours. Mere perforation of the blisters somewhat accelerated the emigration of leukocytes. In contrast, inflammation induced by diphtheria toxoid had a marked effect. The attraction of leukocytes was evident already in one-hour blisters and within 12 hours their total number was not countable. The relative proportions of the inflammatory cells changed during the 12-hour period of inflammation from a neutrophilic to a predominantly mononuclear picture. Eosinophils appeared in the cell population at 4 hours, but their percentage was low and remained rather constant during the study period. Basophils were not found until 12 hours after inflammation had been induced by the toxoid. Then they accounted for two per cent of the total cell population. In contrast, at positive patch test sites the suction vesicles contained basophils in significant amount already one hour after the vesicles were produced. In spongiotic vesicles induced by contact allergens alone, a similar increase in the basophil count is encountered (2).

Suction blistering seems to be mainly a non-inflammatory process. Extravasation of leukocytes was never encountered in the histological or ultrastructural study of biopsy specimens obtained from the base of freshly produced primordial suction vesicles (11). In blisters produced with a suction exceeding −20 cm Hg and in blisters raised on an inflamed skin the fluid may be contaminated with blood. Thus the suction blister technique, adequately controlled, seems to lend itself well to standardized studies of cellular dynamics in different types of inflammation.

SUMMARY

The cellular exudate of intact and incised suction blisters was studied. The inflammatory response was weak, as judged by the number of cells recovered from the blister fluid. The first neutrophils did not appear in intact blisters until after 6 hours; in incised blisters, after 4 hours. A small percentage of lymphocytes appeared after 12 hours. The number of cells was higher in the incised blisters.

Blisters stimulated by diphtheria toxoid showed a stronger inflammatory response and neutrophils were present already after 1 hour; mononuclear cells were predominant after 12 hours. Eosinophils and basophils were then present. In allergic patch test reactions suction induced vesicles before any spontaneous vesicles had appeared, and here basophils were found.

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