

IDENTIFICATION OF MONONUCLEAR CELLS IN CELL SUSPENSION FROM CUTANEOUS INFILTRATES FREED BY A SUCTION BLISTER METHOD

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Abstract. Mononuclear cells were liberated from superficial skin inflammations by a suction blister method which yielded a sufficient number of cells for histochemical and immunocytochemical analysis. The cell population consisted mainly of T cells which were identified as sheep red blood cell rosettes. Very few B cells were found as rosette with immunobeads. Macrophages and eosinophils were found in varying numbers and they were identified histochemically. The method is suitable for studying non-tumorous skin infiltrates.

Key words: Mononuclear cells; Blister fluid; Enzyme histochemical and immunocytochemical study

Very little is known about the mononuclear cells, which predominate in skin infiltrates in inflammatory skin diseases. Only a few diseases have been investigated with modern immunocytochemical or enzyme cytochemical methods. In 1978 the mononuclear cells in Jessner's lymphocytic infiltrate were considered to be B cells (4) but recently they have been shown to be mostly T cells (5). In lichen planus the dermal infiltrate was considered to consist of T lymphocytes (1, 12, 13). These and several other studies are based on sophisticated techniques not available in clinical laboratories.

We have approached the problem of cell typing and T and B cell differentiation by using a cell suspension to provide additional information on the types of inflammatory cells found in superficial inflammations of the skin.

PATIENTS AND METHODS

The patients had two types of inflammatory reactions: persistent plaques of parapsoriasis type (patients 2 and 6) and eczematous reactions (patients 1, 3, 4, 5). All had a round-cell infiltrate in the dermis confirmed by the biopsy taken from the involved abdominal area, where the suction blisters were also generated on the eruptive skin of the abdomen. Detailed data on the patients are recorded in Table I.

The 6 control persons were female hospital staff. During the examination period they showed no signs of clinical

infections or had any medical treatment. Their ages ranged between 22 and 52 years.

Generation and emptying of skin blisters

The suction blister method (8) is based on the generation of small blisters on the skin. A perforated plate with 5 or 6 small holes, each 4 mm in diameter, is placed on the skin of the abdomen. The device is connected to a suction source producing a 150 mmHg vacuum. The skin splits at the boundary between epidermis and dermis. The volume of one blister is about 0.1 cm³. At room temperature the blisters generate in about 1.5-2 hours. On inflamed skin the blisters generate in a shorter time than on healthy skin. It has been proved in practice that blisters cannot be generated on areas of the skin where there are either tumours or heavily inflamed eruptions, because at such sites the blisters burst while splitting out. Depending on the size of the lesion, 12-36 blisters could be generated on the abdominal skin. With the volunteers the blisters numbered from 30 to 36.

The blisters were emptied the next morning by using heparinized Mantoux syringes containing 0.5 ml physiological saline. The contents of the blisters were washed by drawing the fluid 5-8 times back and forth in the syringe and in the blister. After the blisters had been emptied they were broken with a needle and covered with a sterile gauze and kept dry for three days.

Identification of the cells in suspension

The total number of blister cells was determined by the chamber method. One part of the cell suspension was used for cytocentrifuge slides stained with May-Grünwald-Giemsa (MGG), and the other part was used for the enzyme cytochemical identification of lymphocytes and monocyte-macrophages and polymorphonuclear cells *ad modum* Yam et al. (15).

The cells remaining in the suspension were washed three times with cold Dulbecco phosphate-buffered saline (PBS). One part of the suspension was used to determine the percentage of the lymphocytes forming rosettes with sheep red blood cells (E-RFC). Another part was used for determination of the percentage of the cells bearing surface immunoglobulins (SIg cells). The E-RFC-typing was performed *ad modum* Felsburg et al. (7), modified by incubating the pelleted samples at 4°C for 60 min. The E-RFC cells were counted under a coverslip. The result was expressed as a percentage of the lymphocytes. SIg cells were quantitated by membrane immunofluorescence and expressed as a percentage of all blister fluid cells.

Table I. Clinical data of the patients

Patient	Age/sex	Clinical diagnosis	Histological findings from abdominal skin	Additional information
1	50/♀	Erythrodermia Eczema allergicum (Ni, Co)	Perivascular round cell infiltrate eosinophils	Symptoms for 12 months ASTA 16 Epicutaneous tests: Ni++; Co++++ On erythromycin
2	33/♀	Parapsoriasis en plaque	Atrophic epidermis Superficial dense round cell infiltrate	Symptoms for 10 years
3	79/♂	Erythrodermia	Superficial round cell infiltrate, melanin macrophages, some eosinophils	Symptoms for 15 years
4	71/♂	Erythrodermia Alopecia mucinosa	Superficial round cell infiltrate	Symptoms for 2 months No Sézary cells in peripheral blood On erythromycin
5	59/♂	Erythrodermia	Superficial round cell infiltrate penetrating epidermis, some eosinophils	Symptoms for 25 years
6	32/♂	Parapsoriasis en plaque	Dense round cell infiltrate penetrating epidermis, eosinophils	Symptoms for 20 years on abdominal skin; no progress

which contained no keratinocytes. Goat antiserum, specific for the heavy and light chains of each human immunoglobulin class (IgG, IgA and IgM conjugated with fluorescein isothiocyanate, Behring Werke AG, cat. no. OTKG) was used. The third part of the cell suspension was used for the simultaneous counting of lymphocyte subpopulations, T and B cells (9). T cells were identified as E rosette-forming cells (E-RFC) and B cells (IB-RFC) by rosette formation with polyacrylamide gel beads coated with antibody specific to both heavy and light chains of immunoglobulins (Bio-Rad Laboratories; Immunobead, rabbit antihuman immunoglobulin, cat. no. 170-5106). The percentages of IB-RFC and E-RFC were calculated from the total number of lymphocytes.

Heparinized peripheral blood was collected immediately prior to harvesting the blister cells from the patients and the control persons. Total white blood cell count (WBC) and differential count were performed. The immunocytological method used for typing mononuclear cells isolated with Ficoll-Isopaque technique (2) from peripheral blood were the same as those used for blister cell typing.

RESULTS

The total numbers of cells harvested from the blister fluid of the patients varied between 300 and 4800×10^3 . The variation in the number of blister cells from the controls was $1020-2800 \times 10^3$ and seemed to vary within a narrower range than among the patients. There were no keratinocytes or other tissue cells in the suspension.

Figs. 1 and 2 give the results of the MGG and histochemical stainings. Fewer neutrophils were present in the blister fluid of the patients than of the control persons. Mononuclear cells were more prominent in the skin of the patients than in the skin of the controls. Patients 1 and 3 presented with an exceptionally high number of eosinophils, 30.5% and 25%. The number of basophils was low (0-3%)

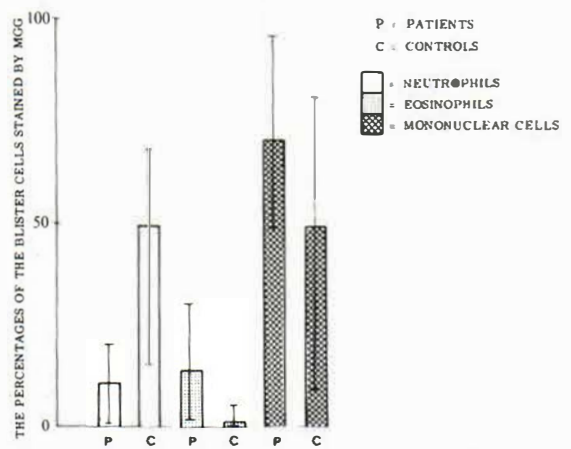


Fig. 1. Columns show the percentage means of the numbers of neutrophils, eosinophils and mononuclear cells in blister fluid. Brackets express ranges (mean \pm standard error).

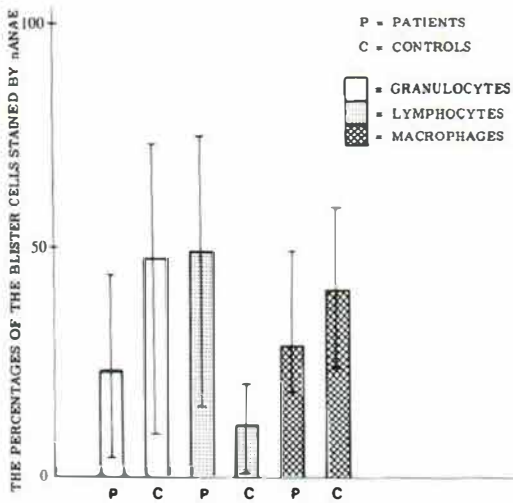


Fig. 2. Columns show the percentage means of granulocytes, lymphocytes and macrophages stained by nANAE. Brackets express ranges (mean \pm standard error).

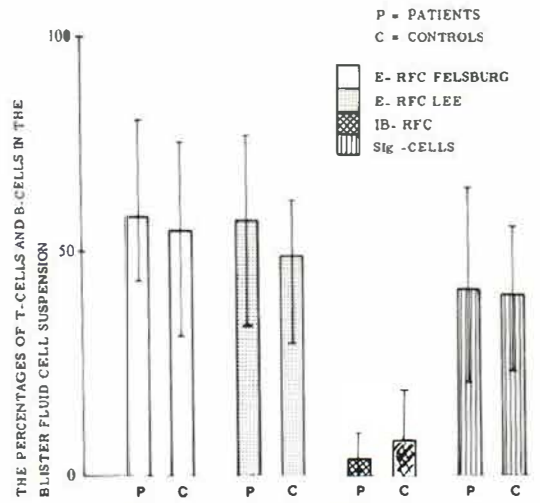


Fig. 3. Columns show the percentage means of T, B and SIg cells according to different methods. Brackets express ranges (mean \pm standard error).

in all counts. Using an enzyme cytochemical method, it was shown that the percentage of macrophages varied from 16 to 40 among the patients and from 24 to 58 among the controls. The percentage of macrophages was higher in the blister fluid of the control persons than of the patients, whereas the percentage of the lymphocytes was greater (30–68%, \bar{x} = 49%) in the blister fluid of the patient as compared with the controls (3–20%, \bar{x} = 11.7%).

The percentages of T cells from the lymphocytes were similar with both techniques (Fig. 3), and there was no marked difference between the patients and control persons, although the total of lymphocytes in the cell suspension of the controls was smaller (Fig. 2). Patients 2 and 6 had the highest percentages of lymphocytes (76% and 62%). The percentages of the T cells of their blister fluid lymphocytes were also high (66% and 56% according to Felsburg, or 77% and 57% according to Lee).

The number of B cells counted as Immunobead rosette-forming cells (IB-RFC) was slightly lower in the patients than in the control persons.

SIg cells were numerous. In addition to B cells, there were macrophages and granulocytes, which can bind the fluorescein-conjugated antiserum with their Fc receptors. No differences were found between the SIg cells of the patients and of the controls.

Total white blood cell counts and total numbers of lymphocytes in peripheral blood of both patients and control persons were within normal ranges. The percentages of E-RFC and SIg cells or IB-RFC were similar in both groups, as can be seen in Table II.

Table II. Percentage of T cells, SIg cells and B cells (IB-RFC) from peripheral blood. Mononuclear cells were isolated by Ficoll-Isopaque technique ($10^6/l$)

	Leuk. ($10^6/l$)	Lymph. (%)	Lymph. ($10^6/l$)	E-RFC ^a (%)	E-RFC ^a ($10^6/l$)	IB-RFC ^a (%)	SIg cells ^a (%)
<i>Patients</i>							
Mean	7 600	30	2 200	78	1 700	3.5	18
Mean \pm SD	5 500–9 700	19–41	1 400–3 000	74–82	1 100–2 300	2–5	12–24
<i>Controls</i>							
Mean	5 300	46	2 400	77	1 875	4.7	14
Mean \pm SD	4 400–6 200	41–51	1 900–2 900	66–88	1 375–2 375	3–6	12–16

^a Isolated by Ficoll-Isopaque technique.

DISCUSSION

Mononuclear cells infiltrating the skin in various disorders have been characterized in frozen tissue sections (5, 6, 10, 11) and in mechanically disaggregated (4, 5, 14) or enzymatically liberated cell suspensions (12).

In our study the cell suspension was obtained directly from the inflammatory skin and contained a sufficient number of cells for several types of analysis. This is an advantage of our method compared with those in which the size of the biopsy must exceed 1 000 mg to provide a sufficient amount of tissue for the mechanical disaggregation (3). On the other hand, the suction blisters require an even surface and are not suitable for tumourous infiltrates.

Two patients (numbers 2 and 6) had longstanding plaques of parapsoriasis without atypical cells. In these patients the cell suspension differed from that of the other 4 patients with an eczematous histological picture and erythrodermic clinical inflammation. Their cell suspension consisted almost exclusively of mononuclear cells with a few polymorphonuclears, and in the other case eosinophils. The percentage of lymphocytes was also highest in these cases, which means that the infiltrate consisted almost exclusively of T lymphocytes. It is well known that lesions of this type are potentially malignant and that the possible malignancy is mycosis fungoides, which is considered to be a T cell lymphoma.

In the erythrodermas, the proportions of eosinophils, lymphocytes and macrophages varied, but in every case the majority of the lymphocytes were T lymphocytes.

The proportion of the polymorphonuclears tended to be lower in the patient group than in the control group. The complete absence or presence of only a small number of eosinophils was conspicuous in the control group. This shows that the damage caused by generating the blisters in healthy skin is different, and probably simpler, compared with the various inflammatory stimuli effective in the lesional skin. It is also possible that the inflammation induces agents inhibiting the mobility of the polymorphonuclears and eosinophils.

The figures for T cells and B cells never add up to 100% in the blister fluid of the patients or of the controls. This might indicate that the blister fluid prevents the expression of the surface markers.

Another possibility is that the cell suspension contains partly null cells and plasma cells or passenger cells without a specific function and identification.

The present study shows that the blister fluid cell suspension provides one possibility of examining tissue cells with modern techniques without damaging the cells. The results also indicate that the most important reactive pattern consists of T cells and macrophages in eczematous reactions, and of T cells in parapsoriasis en plaque. The mechanical destruction of epidermis in blister generation induces mostly the mobility of polymorphonuclears and macrophages in healthy skin.

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Received December 18, 1981

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