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REPORTS

T-CELL MEMBRANE CHARACTERISTICS OF "MYCOSIS CELLS" IN THE SKIN AND LYMPH NODE

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In some patients with mycosis fungoides atypical cells ("mycosis cells") are found in the blood. Recently the T-cell membrane characteristics of these atypical cells have been described. In this paper the results of a study of the atypical cells isolated from the lymph nodes and the skin lesions of three patients with mycosis fungoides are presented. Using electron microscopy, it could be demonstrated that the atypical cells formed rosettes with uncoated sheep red blood cells, but not with antibody–complement-coated sheep erythrocytes, indicating the T-cell membrane characteristics of the atypical cells.

There are many similarities and also a few differences in morphology between the atypical cells of the Sézary syndrome and those of mycosis fungoides as described by Lutzner et al [1]. It has recently been stated that the atypical cells in the blood of patients with either Sézary syndrome or mycosis fungoides have T-cell membrane characteristics, as studied by means of E-rosette formation [2–4] and specific rabbit antihuman T-cell sera [2]. However, in contrast to the Sézary syndrome, most patients with mycosis fungoides have few or no atypical cells in the blood [1]. Therefore it seemed more rewarding to study the membrane characteristics of the "mycosis cells" in other tissues and especially in the skin lesions, in which they can occur in abundance.

Although Edelson et al [5,6] state that the majority of the cells isolated from the skin of patients with mycosis fungoides formed E-rosettes and that there was no evidence that the cells in the dermal infiltrate were B cells or histiocytes, they did not mention the use of electron microscopy for the identification of the cells in the rosettes. In this paper, direct evidence is presented that some of the cells having mycosis cell morphology and occurring in the skin lesions of two of our patients and in a lymph node of another patient, form E-rosettes.

MATERIALS AND METHODS

Patients. Patient B.D., an 80-year-old male, had skin lesions consisting of erythematous, slightly infiltrated plaques on the trunk for 5 years before admission in December, 1974, to the Department of Dermatology, University Medical Centre, Leiden. These plaques gradually developed into tumors, up to 5 cm in diameter, on both legs and arms and on the back. The patient showed enlarged inguinal lymph nodes. The blood leukocyte count was normal (8400/mm^3, lymphocytes 12%, monocytes 6%) and no atypical cells could be demonstrated.

Patient A.S., a 62-year-old man, had skin lesions for 6 years, and on admission a few tumors up to 4 cm in diameter were found on his chest and back. No enlarged lymph nodes were palpable. Blood leukocyte count was normal (5700/mm^3, lymphocytes 30%, monocytes 3%) and atypical cells were not found.

Patient G.J., a 57-year-old man, had minor skin lesions for 2 years. On admission some infiltrated plaques were found on his chest. There were no enlarged lymph nodes palpable. Blood leukocyte count was normal (6500/mm^3, lymphocytes 26%, monocytes 4%), without atypical cells.

In all three patients the diagnosis of mycosis fungoides was made on clinical and histopathologic criteria [7,8], the latter being a dense dermal infiltrate containing atypical mononuclear cells with large hyperchromatic indented nuclei (mycosis cells) and varying numbers of eosinophils, lymphocytes, and monocytes. Infiltration of the epidermis had occurred but Pautrier's microabscesses were not found. According to the accepted criteria, patients B.D. and A.S. were both in stage 3 (infiltrates throughout the dermis), whereas patient G.J. was in stage 2 of the disease (band-shaped infiltrate). From all three patients skin biopsy material and blood samples were processed for electron microscopy. The rosette tests were performed on the mononuclear cells isolated from a lymph node of patient B.D., as well as on the mononuclear cells from the skin lesions of patients A.S. and G.J. Rosette tests were also done on the mononuclear cells isolated from the blood of all three patients.

Isolation of mononuclear cells from the blood. Freshly drawn heparinized venous blood was diluted with an equal volume of phosphate-buffered saline (PBS) at pH 7.4 and layered on a Ficoll-Isopaque mixture (d = 1.077 gm/cm^3) at 20°C. After centrifugation at 1,000 × *g* for 20 min, the cells at the interphase were harvested and washed twice with PBS. No attempt was made to eliminate the monocytes from the isolated mononuclear cell population. (The percentage of monocytes in our isolated cells can be estimated at 6 to 15%).

Cell isolation from skin and lymph node. The speci-

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mens of the skin and the lymph node were thoroughly minced in RPMI 1640 medium (Flow Laboratories). The cell suspension was filtered through nylon gauze. The cells were spun down and washed 3 times with PBS (pH 7.4). The viability tested with trypan blue was always greater than 90%.

Preparation of normal erthrocytes (E), antibody-coated erythrocytes (EA) and antibody-complement-coated erythrocytes (EAC). Sheep red bloods cells (SRBC) were stored in Alsevers solution at 4°C and used between 1 and 3 weeks after collection. Uncoated cells (E) were prepared by washing the SRBC 5 times with PBS (pH 7.4).

For the preparation of EAC, rabbit-antisheep hemolysine (Difco) (1:4,000) and, as the source of complement, fresh C_3H mouse serum (1:10) were used. In later experiments, IgM rabbit-antisheep hemolysine (1:2,000) was used (provided by Dr. W. P. Zeylemaker, Central Laboratory of the Netherlands Red Cross blood transfusion service).

Procedures for the preparation of rosettes. The E-, EA-, and EAC-rosette tests on mononuclear cells were performed according to the methods described by Zeylemaker et al [9]. All tests were carried out in duplicate. A conglomerate of a cell to which 3 or more SRBC adhered, was scored as a rosette. The percentage of rosettes was estimated by staining the suspension with brilliant cresyl blue (Gurr, High Wycombe, England), and counting 100 mononuclear cells in a hemacytometer.

In the EA-rosette and EAC-rosette procedures, the cell suspension was spun down and, after rosettes had formed in the pellet, they were resuspended by vigorous shaking for 15 sec on a Vortex mixer. It could be demonstrated that the latter procedure completely disrupts the E rosettes. The EA background never exceeded 1%.

Processing of rosettes for electron microscopy. Suspensions containing rosettes were fixed in 2% cacodylate-buffered glutaraldehyde at 4°C for 1 night. After postfixation in 2% veronal acetate-buffered osmium tetroxide for 2 hr and dehydration in ethanol, the specimens were embedded in Epon 812. Ultrathin sections were stained with uranylacetate and lead citrate.

RESULTS

Contrary to the findings in the skin lesions and in the lymph node, in which the mycosis cells were numerous, no atypical cells were found in the blood samples taken from the three patients. Electron microscopically, the morphology of the mycosis cells corresponded well with the descriptions of Lutzner et al [1] and Rosas-Uribe et al [10] (Fig. 1).

The results of the E-rosette and EAC-rosette tests on isolated mononuclear cells from the blood, the lymph node, and the skin are given in the Table.

Electron microscopy of rosettes, prepared according to the E-rosette procedure on cells isolated from the lymph node and the skin, showed some variation in the type of the central cell. Mononuclear cells were found in all instances, most of them showing the morphologic characteristics of lymphocytes. No monocytes or polymorphonuclear leukocytes were ever seen in the rosettes. It is of interest that, in some of these rosettes, cells with the morphologic characteristics of mycosis cells were found (Figs. 2, 3). It should be noted, that some of the mycosis cells had apparently failed to associate with the SRBC (Fig. 1).

Large numbers of rosettes, prepared according to the EAC-rosette procedure on cells isolated from the same tissues, were also studied with the electron microscope. It was a constant finding that the majority of the central cells had the morphologic characteristics of monocytes and a few those of lymphocytes, but that cells with mycosis-cell characteristics were invariably absent.

DISCUSSION

We have studied the membrane characteristics of the mycosis cells derived from the skin lesions of two patients and from lymph-node material of another patient with mycosis fungoides. Technically, the number of cells in the dermal infiltrate seems to correlate with the ease with which cells can be isolated from the skin specimens. From small infiltrates, few cells (2.5×10^6) can be harvested (patient G.J.).

Using electron microscopy, we could identify mycosis cells in some of the E-rosettes of cells de-

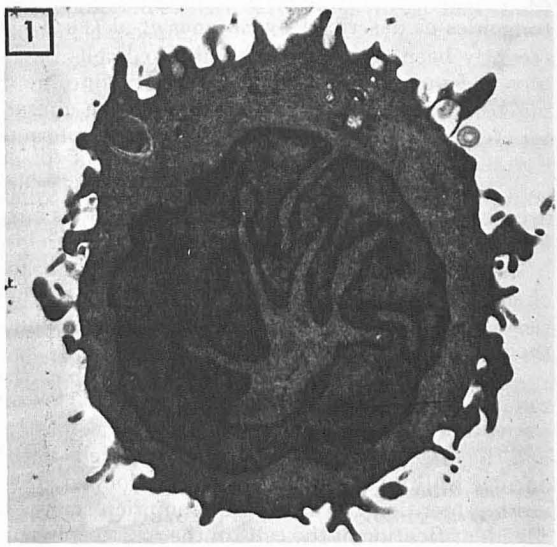

FIG. 1. Electron microscopy of a "mycosis cell" from the lymph node of patient B.D. This cell failed to bind SRBC in E-rosette procedure (approx. 7500×).

TABLE *Percentage of mononuclear cells forming rosettes*

Patient	Stage	Skin		Blood		Lymph node	
		E	EAC	E[a]	EAC[a]	E	EAC
B.D.	3	NT	NT	55	50	47	NT
						36[b]	26[b]
A.S.	3	51	29	62	42		
G.J.	2	60	15	69	33		

[a] Normal values (n = 12) E: 59.2 ± 6.3; EAC: 32.1 ± 4.1.

[b] After freezing in liquid nitrogen.

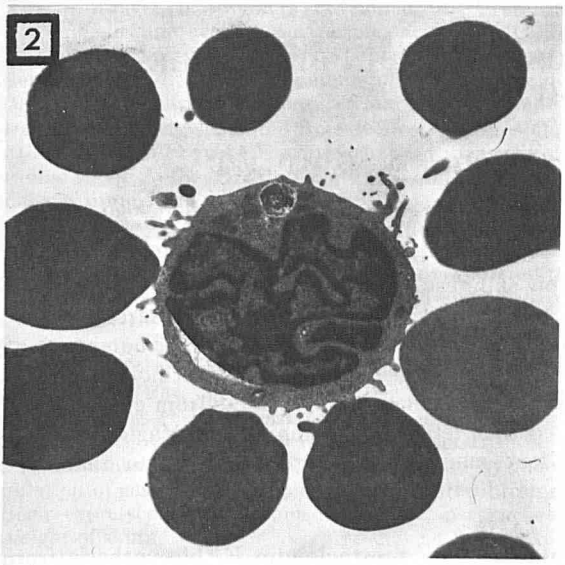

FIG. 2. E-rosette around an atypical cell, isolated from the lymph node of patient B.D. (approx. 5000×).

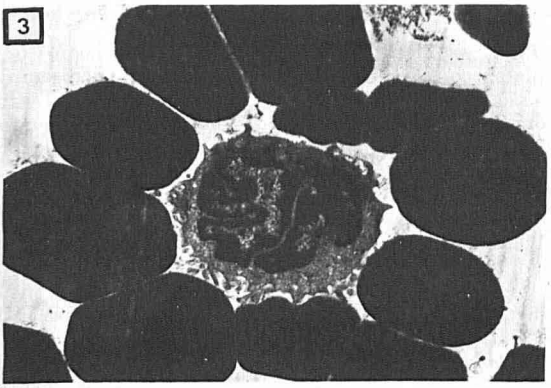

FIG. 3. "Mycosis cell" from the skin of patient A.S. in the center of an E-rosette (approx. 5200×).

rived from skin specimens and lymph-node material. This direct evidence brings additional information on the findings of Edelson at al [5,6] concerning the nature of the cells in the skin lesions of three patients with mycosis fungoides. It is interesting that Edelson et al [5,6] and Robinowitz et al [11] found a higher percentage of E-rosettes on mononuclear cells, isolated from the dermal infiltrate, than we did. One possibility is that this difference can be explained by the stage of the disease at which the biopsy material was taken. However, this discrepancy could also be the result of differences in the techniques used. Edelson et al [5] used neuraminidase-treated SRBC and his rosette tests were performed in 100% fetal calf serum. Both these procedures are known to result in higher percentages of E-rosettes [12,13]. In our percentages of EAC rosette-forming cells, monocytes are included. This can explain the relatively high percentages of the EAC rosette-forming cells in the peripheral blood as compared with the findings of Edelson [5,6] and Robinowitz [11].

Concerning the composition of the dermal infiltrate, patient A.S. is of interest because in our investigation the dermal infiltrate contained cells with a complement receptor, as is found on B cells and monocytes. Edelson et al [5,6] could not find indications that cells in the skin lesions of mycosis fungoides had complement receptor sites. The electron microscopic examination of the E rosettes showed that some mycosis cells failed to bind SRBC. Zucker-Franklin et al [4] described the same phenomenon for the atypical cells in the peripheral blood of patients with the Sézary syndrome.

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