CONCISE REPORT

Capping and Freeze-Fracture Analysis of Sézary Cells

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The functional integrity of blood and skin Sézary cells was examined in regard to motility, capping proficiency, and intramembranous particle (IMP) mobility. In contrast with chronic lymphocytic leukemia lymphocytes, which are relatively inert in these respects, variable proportions of Sézary cells are motile and are able to cap and cluster their IMPs. Since these functions may reflect a cell's propensity for motility, capping proficiency, and intra- diapedesis, these observations may help to explain the inverse relationship between the size of the skin infiltrate and the number of circulating Sézary cells often observed in this condition.

EXPERIMENTS designed to define the cytoskeletal and membrane abnormalities in chronic lymphocytic leukemia (CLL) cells have shown that the failure of these cells to cap is accompanied by a lack of uropod formation, a dearth of intermediate (10-nm) filament polymerization, and decreased mobility of the intramembranous particles (IMP), as revealed by freeze-etching. Since these defects could not be attributed to the B-cell nature of CLL cells, it seemed of interest to explore whether or not neoplastic lymphocytes obtained from patients with other lymphoproliferative diseases have similar abnormalities. Patients with the leukemic variant of mycosis fungoides (the Sézary syndrome), whose circulating lymphocytes are known to represent neoplastic T cells, seemed appropriate for this study. Accordingly, the cells of 3 patients with the Sézary syndrome and the lymphocytes infiltrating the skin of a fourth patient without blood involvement were observed in the living state by phase microscopy and following treatment with a membrane cross-linking ligand by fluorescence and electron microscopy. The studies showed that in contrast to CLL lymphocytes, many Sézary cells were able to cap the attached ligand, and showed parallel rearrangement of 10-nm filaments between the nucleus and the pole. In addition, glycerol treatment prior to fixation caused aggregation of IMP, a phenomenon not seen in CLL lymphocytes.

MATERIALS AND METHODS

Heparinized blood was obtained from 3 patients with the Sézary syndrome whose absolute lymphocyte counts ranged from 24,000 to 64,000/cu mm and whose skin biopsies were diagnostic of mycosis fungoides (MF). The lymphocytes were purified on Ficoll-Hypaque gradients and subsequent removal of monocytes with iron filings as described previously. The specimens selected for this study consisted of more than 70% Sézary cells. The terms MF and Sézary cell are used interchangeably. The cells obtained from the biopsy were teased from the specimen with a needle and treated in suspension like the blood cells. Ultrastructurally, 85% of these cells were MF cells by criteria defined elsewhere and had the
surface properties of the T-cell class of lymphocytes. The lymphocytes of 20 normal subjects and 22 patients with CLL, prepared identically, were the subject of a previous report. The Ig fraction of a rabbit antilymphocyte serum (ALS-Ig) kindly provided by Dr. L. Liebes was isolated and coated onto 0.1-μm latex spheres as described in detail elsewhere. Aliquots of $10^9$ cells in 0.7 ml of Hanks' saline and 0.3 ml of the Ig-coated particles (ALS-Ig-P) maintained at 37°C were observed by phase microscopy at intervals ranging from 10 to 60 min. Aliquots were also prepared for electron microscopy by methods routine in this laboratory. For freeze-fracture studies the cells were placed into 25% glycerol for 2 hr at

Fig. 1(A.) Sézary cell obtained from peripheral blood fixed in suspension. The uropod shows many villous processes. Note the deep cytoplasmic invaginations into the nucleus and the prominent nucleolus (Nu). $\times 9000$. (B) Various aspects of Sézary cells treated with ALS-Ig-P at 37°C for 30' min prior to fixation. Note the large nucleoli (Nu). The pole of the cell bearing the antibody-coated particles (L) displays numerous cellular processes and in fortuitous sections deep invaginations (arrow in D). B: $\times 9600$, C: $\times 9000$, D: $\times 8000$. 

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20°C prior to fixation with glutaraldehyde, or they were fixed before cryoprotection with glycerol. Freeze-fracture was carried out with a Balzer high-vacuum freeze-etch unit (BAF-300) as before. Thin sections and freeze-etched replicas were examined in a Siemens Elmiskop electron microscope.

RESULTS

Examination of living Sézary cells by phase microscopy showed that 10%-15% of the cells were motile and extended uropods whether or not they were treated with ALS-Ig. An electron micrograph of such a cell is shown in Fig. 1A. After treatment of the cells with ALS-Ig-P at 37°C for 5 min, almost all the cells were covered with particles, giving a patched appearance on phase microscopy, as was the case for normal lymphocytes and CLL cells. Uncoated latex particles do not adhere to Sézary cells. After 30 min of incubation at 37°C, variable percentages of the blood Sézary cells ranging from 3% to 15% showed displacement of the particles to one pole, i.e., cap formation. In the specimen prepared from the skin infiltrate, caps were seen on more than 50% of the Sézary cells. On electron microscopy, the region of the cell associated with the cap appeared similar to that of normal capped lymphocytes, showing numerous invaginations and villous processes (Fig. 1B-D). At higher resolution (Fig. 2), the following cytoskeletal structures were identified: (1) a meshwork of actinlike microfilaments (7 nm) located predominantly in the submembranous cytoplasm; (2) thick bundles of intermediate filaments (10 nm) that were oriented parallel to the long axis of the uropod and did not appear to reach the plasma membrane; (3) innumerable microtubules (25 nm) generally having the same orientation as the intermediate filaments and also failing to reach the plasma membrane.

Replicas of two freeze-fractured Sézary cells are shown in Fig. 3. The nuclear pores aided in the recognition of the nuclear membrane and supported the impression gained from thin sections that the nucleus was furrowed by narrow cytoplasmic invaginations rather than being folded upon itself. Glycerol treatment prior to fixation caused clustering of intramembranous particles (Fig. 3A, inset), as had been demonstrated for normal lymphocytes.

DISCUSSION

Interest in Sézary cells became widespread when the Sézary syndrome was the first human lymphoproliferative disorder to be recognized as a T-cell neoplasm. Despite the low prevalence of the syndrome or the related entity MF, the Sézary cell has continued to draw attention for several reasons: (1) its origin is still unknown; (2) the question whether or not the disease is due to clonal expansion of a lymphocyte subclass that undergoes malignant transformation during the course of the disease has not been settled; (3) the functional integrity of the cells in regard to locomotion, the ability to form caps, and the potential to respond to antigenic stimuli is still in doubt.

These studies have demonstrated that variable proportions of Sézary cells remain motile and able to cap, whereas other morphologically identical cells appear to be inert in these respects. This finding underscores earlier observations that the numbers and surface properties of Sézary cells may change with time even in the absence of therapy. It also implies that the morphologic aberration should not be equated with altered function or malignant transformation. The functional heterogeneity may account for the divergent results obtained in different laboratories.
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Fig. 2. Inset shows a Sézary cell that had been incubated with ALS-Ig-coated latex particles for 30 min at 37°C. A cap is seen over the uropod. Note that the cerebriform nucleus does not appear to be influenced by the change in contour of the cell. ×3000. The uropod of the cell depicted in the inset is seen at higher resolution. Note that the microtubules (MT) and 10-nm intermediate filaments (IF) appear to run mostly in parallel bundles, whereas the actinlike microfilaments (MF) form a submembranous network. ×38,000.
Fig. 3. Freeze-fracture replica of a Sázary cell that had been treated with glycerol before fixation. This treatment caused tight clustering of the IMPs in the plasma membrane. The arrow indicates an area with clusters illustrated at higher magnification in the inset. The nuclear pores (P) are helpful in delineating multiple planes through which the nucleus has been cleaved. C: cytoplasm. x 15,000; inset. x 30,000.

Fig. 4. Freeze-fracture replica of a Sázary cell showing the extremely narrow cytoplasmic invaginations (C) that almost divide the nucleus into separate compartments. Many facets of the nuclear membrane can be appreciated by means of the nuclear pores (P). x 17,000.
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when responses to mitogens or in vitro cellular immunity were examined.7-9 When
capping occurred, the ultrastructural concomitants (i.e., microtubule and 10-nm
filament reorientation) were identical to those in normal cells.1 This suggests that
the intermediate filaments, which form more conspicuous organelles in Sézary cells
than in normal lymphocytes,7 may subserve a normal function. The freeze-fracture
observations have extended studies by others who pointed out that such analyses are
helpful in distinguishing cytoplasmic invaginations from a twisted nucleus.10 In
addition, the present manipulations have shown that the IMPs in the plasma
membrane of Sézary cells are readily relocated by glycerol treatment prior to
fixation. Free mobility of IMPs into tight clusters as a consequence of such in vitro
treatment has been observed in many normal cells, including lymphocytes,11 but has
not occurred in CLL cells under similar conditions.1 The finding of an inordinately
high percentage of motile and capped cells in the skin of 1 patient who had very few
Sézary cells (>5%) in his blood suggests that the functional integrity of the cells
may determine the degree of diapedesis and tissue infiltration. Unfortunately, the
number of cells that can be prepared from skin biopsies obtained for diagnostic
purposes is usually too small to permit the complete morphologic and functional
analyses necessary to prove this hypothesis.

REFERENCES

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