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Ultrastructural, Immunologic, and Functional Studies on Sézary Cells: A Neoplastic Variant of Thymus-Derived (T) Lymphocytes

(mycosis fungoides/thymus-derived (T)-cell rosette/lymphoid neoplasms)

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ABSTRACT The vast majority of human lymphoid neoplasms examined to date have been associated with a proliferation of bone marrow-dependent (B) lymphocytes. In an effort to delineate human tumors of T-cell (thymus-dependent) lineage, use was made of the peripheral blood leukocytes of sixteen subjects with various forms of mycosis fungoides. The abnormal cells in the circulation of these patients are morphologically identical to those that infiltrate their nodes and skin. On electron microscopy, such neoplastic lymphocytes (Sézary cells) had "cerebriform" nuclei and an abundance of cytoplasmic fibrils not described heretofore. Sézary cells were nonadherent and nonphagocytic and usually responded to stimulation with phytohemagglutinin, refuting earlier suggestions that the cells represent monocytes or histiocytes. In contrast to chronic lymphocytic leukemia lymphocytes, the Sézary cells lacked surface immunoglobulin and receptors for complement. Ultrastructural analysis identified Sézary cells in the center of directly formed rosettes (E-rosettes) characterizing the behavior of T lymphocytes in this test. Though some Sézary cells lacked both T and B cell-surface properties, in general, these observations support the view that the Sézary cell is a neoplastic variant of a thymus-derived lymphocyte.

It is curious, that in man, in contrast to most rodents, the vast majority of chronic lymphocytic leukemias studied to date, have been associated with an increased level of circulating bone marrow-derived (B) lymphocytes (1–7). Such cells carry immunoglobulins on their surface (8, 9), possess membrane receptors for immunoglobulin (10, 11) and complement (12), and respond poorly to phytohemagglutinin (PHA) *in vitro* (13–15). Though it is conceivable that plasma cells and B-lymphocytes are more susceptible to mutagenic change because of their physiologic responsiveness to antigenic stimuli, it was to be anticipated that human neoplasms of thymus-derived lymphocyte (T-cell) origin would also be found.

Indeed, the Sézary syndrome, a variant of mycosis fungoides in which peculiarly shaped cells infiltrate the skin, nodes, as well as circulating blood, confirmed this prediction. Preliminary studies showed that Sézary cells lacked B-cell surface markers and that they had properties of T-lymphocytes (16, 17). The present report concerns a more extensive analysis of the cells obtained from eight patients with the diagnosis of Sézary syndrome and eight patients with the diagnosis of mycosis fungoides. In addition to the convoluted nucleus, electron microscopic studies showed that most Sézary cells contain a network of cytoplasmic fibrils not reported heretofore. To minimize the possibility that such cells are monocytes or histiocytes, we demonstrated that Sézary cells are neither adhesive nor phagocytic. The cells of most patients responded to PHA stimulation *in vitro*, all cells lacked B-cell surface markers, and the majority of Sézary cells, but not all, formed direct rosettes with sheep erythrocytes, suggesting that they represent T-lymphocytes.

Abbreviations: B- and T-lymphocyte, bone marrow- and thymus-derived lymphocyte, respectively; PHA, phytohemagglutinin.

MATERIALS AND METHODS

Patients. The diagnosis of mycosis fungoides was established by skin biopsy in all subjects. Sézary syndrome was the eponym used when the patient had generalized erythroderma, lymphadenopathy, and Sézary cells recognized on routine blood smears (18). Skin lesions had been present from 3 months to 30 years. Except in one instance, systemic therapy had been discontinued for at least 1 month before the studies.

Cell Preparation. Fifty to 100 ml of heparinized venous blood were centrifuged at $200 \times g$ for 5 min. The mononuclear cell fraction was prepared from the plasma and buffy coat by centrifugation on a Ficoll–Hypaque mixture (Pharmacia, Uppsala, Sweden and Winthrop Laboratories, N.Y., respectively) essentially as described by Boyum (19). Aliquots of this preparation were washed twice in Hank's solution and used for phagocytosis and electron microscopy. For the remainder of the studies, the mononuclear cell suspensions were purified further by magnetic removal of monocytes after incubation of the cells with iron filings, as described (20). This will be referred to as the purified lymphocyte fraction.

Phagocytosis. Before and after removal of monocytes, 5×10^6 washed cells were resuspended in 3 ml of Hank's solution containing 20% autologous plasma and 30 μl of latex particles (Dow Chemical Co., Midland, Mich.) 0.3 μm in diameter. The suspensions were incubated on a rotating tumbler for 20 min at 37° before fixation for electron microscopy.

Fluorescence Microscopy. Supravital immunofluorescence studies were carried out on purified lymphocytes, that had been washed three times, by utilizing polyvalent fluorescein-conjugated rabbit antiserum to human immunoglobulin essentially as described (21).

Complement Receptor (B) Lymphocytes (EAC-Rosettes). The percentage of lymphocytes with complement receptors was quantitated essentially as by Bianco *et al.* (22). Trypsinized sheep erythrocytes (E) coated with rabbit antisheep hemolysin (GIBCO, Grand Island, N.Y.) and CF1 mouse serum as a source of complement were prepared by mixing equal volumes of a 2-mg/ml of solution of trypsin (Calbiochem., LaJolla, Calif.) with 5% erythrocytes in RPMI 1640. After incubation for 1 hr at 37°, an equimolar concentration of soybean trypsin inhibitor (Calbiochem., LaJolla, Calif.) was added and the cells were washed three times.

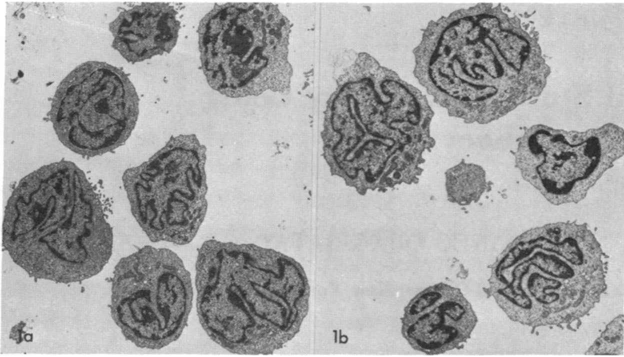

FIG. 1 (*a* and *b*). Low-power survey electron photomicrographs of purified lymphocytes from the blood of patients M.P. and P.P., respectively. Typical Sézary cells showing "cerebriform" nuclei are readily apparent, but the fibrillar structures are not resolved at this magnification. Magnification ×2200.

Sheep Erythrocyte Receptor (*T*) *Lymphocytes* (*E-Rosettes*). The percentage of T-cells on the basis of the number of rosettes formed with sheep erythrocytes was determined by a modification of the method of Lay *et al.* (23). A 5% suspension of sheep erythrocytes in Hank's solution (pH 6.5) was treated for 1 hr at 37° with 0.2 unit of neuraminidase (type IV from *Clostridium perfringens*, Sigma Chemical Co., St. Louis, Mo.) that had been dissolved in 0.2 ml of Hank's solution. The lymphocyte and sheep erythrocyte mixture was incubated for 15 min at 37°, centrifuged at 200 × *g* for 10 min at 25°, and placed on ice for 15 min. Only lymphocytes with three or more erythrocytes were counted as rosettes.

PHA Stimulation. Triplicate 4-ml cultures of purified lymphocytes containing 10^6 cells per ml in RPMI 1640 with 20% fetal-calf serum (GIBCO, Grand Island, N.Y.) were set up with or without 0.1 ml of RPMI 1640 containing serial 10-fold dilutions of PHA (Wellcome, Beckenham, England). Twenty hours before cells were harvested, 0.1 μCi of a solution of [*2*-^{14}C]thymidine (specific activity 55.7 mCi/mmol, New England Nuclear Corp., Boston, Mass.) in 0.1 ml of RPMI 1640 was added to each tube. The cells were processed, counted as described (24), and/or smeared and stained to assess the percentage of transformed cells.

Electron Microscopy. The following specimens were fixed at room temperature or at 4° in 3% phosphate-buffered glutaraldehyde overnight: (*1*) unwashed buffy coat cells; (*2*) mononuclear cells from Ficoll-Hypaque gradients before and after incubation with latex particles; (*3*) purified lymphocytes; (*4*) E-rosettes; and (*5*) PHA-stimulated cells. Post-fixation with 2% osmium tetroxide and embedding in Epon 812 were carried out as described (21). The percentage of Sézary cells was counted in specimens from which monocytes had been

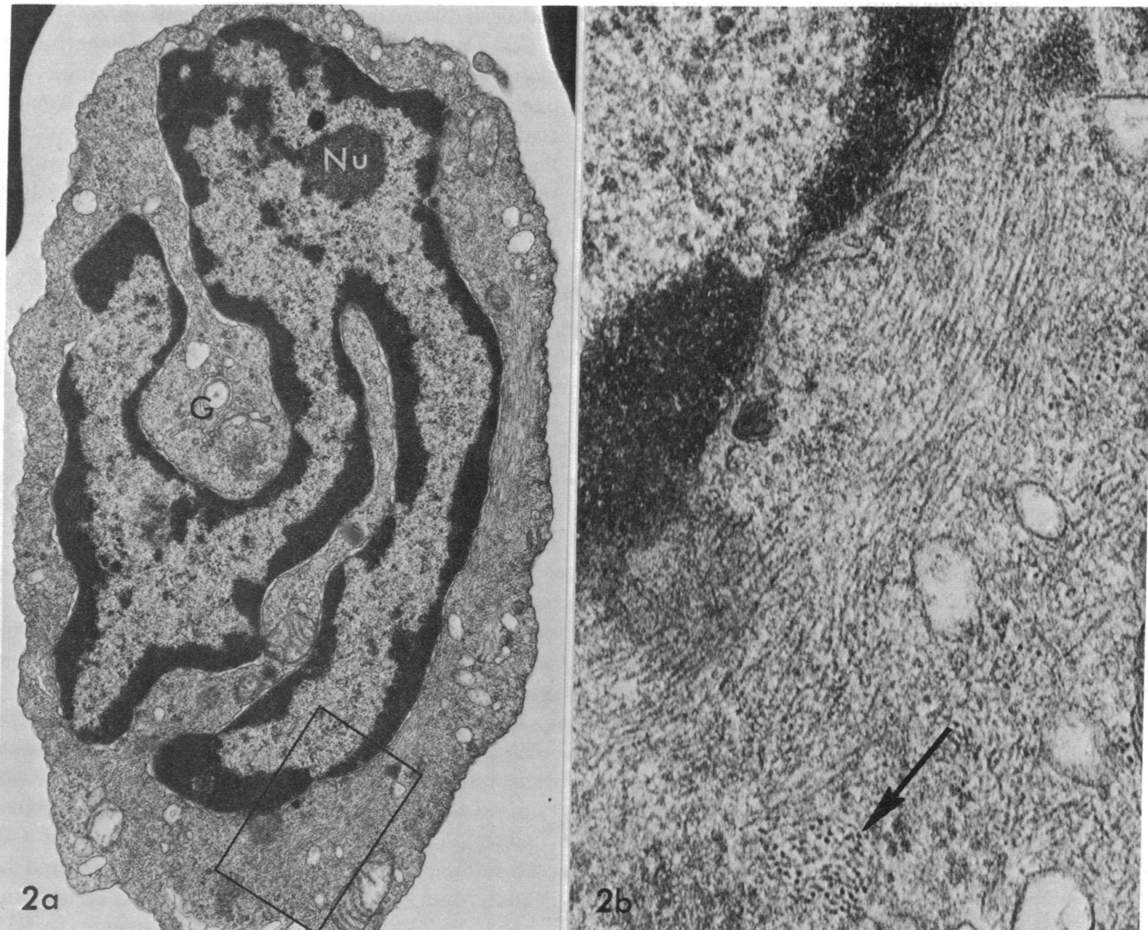

FIG. 2. (*a*) Typical Sézary cell showing a convoluted nucleus and prominent nucleolus (*Nu*). The entire cytoplasm is filled with fibrils, difficult to see at this resolution. The area within the *rectangle* is shown at higher magnification in *b*. *Nu*, nucleolus; *G*, Golgi zone. Magnification ×12,500. (*b*) Higher magnification of the area demarcated by the *rectangle* in *a*. Fibrillar structures appear to crisscross within the cytoplasm. They are seen in longitudinal section parallel to the nuclear membrane, and in cross section at *arrow*. Magnification ×60,000.

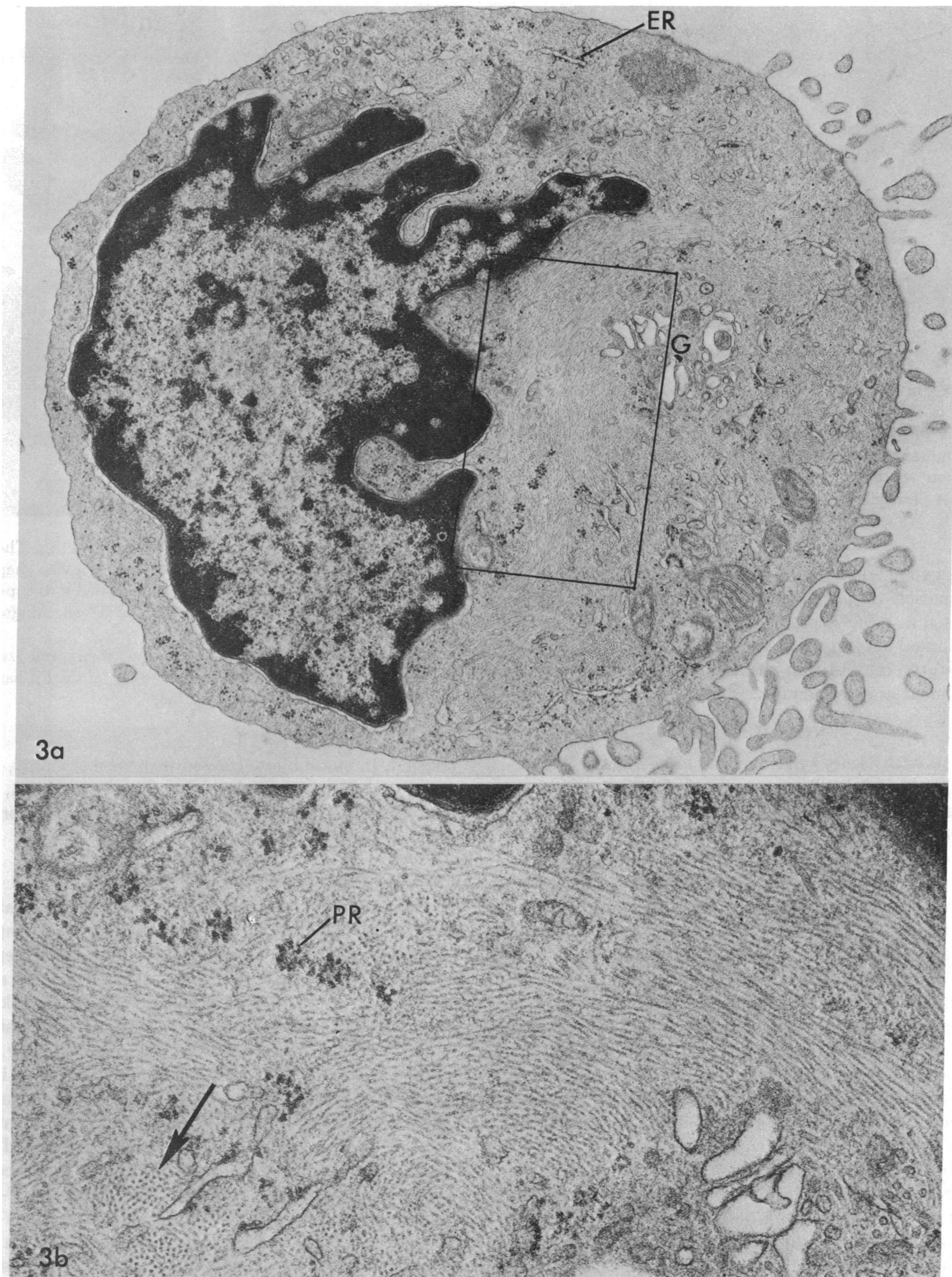

FIG. 3. (*a*) Sézary cell showing five cytoplasmic indentations into tangentially sectioned nucleus. Many thin processes appear at one pole of cell opposite the Golgi zone (*G*). The cell has very little rough endoplasmic reticulum (*ER*), but prominent clusters of polysomes. The fibrillar structures are seen throughout the cytoplasm. The *demarcated area* is illustrated at higher magnification in *b*. Magnification ×21,000. (*b*) Higher magnification of the area demarcated by the *rectangle* in *a*. *PR*, polyribosomes. *Arrow* pointing to small dots indicates fibrils in cross section. Magnification ×70,000.

eliminated. Thin sections were mounted on slit grids so that an entire section could be viewed at magnifications of 600–800. A minimum of 500 cells in three different block "faces" were counted per specimen. As a rule, the convoluted nucleus of a Sézary cell (Figs. 1–3) was easily distinguished from that of a normal lymphocyte. However, since a thin section may not pass through the most optimal plane of the nucleus, some arbitrary criteria were established. Only lymphocytes with

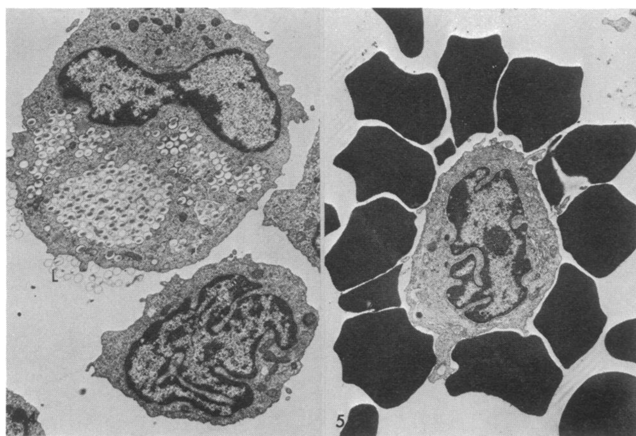

FIG. 4. Cells obtained from a Ficoll-Hypaque gradient without further purification were incubated with latex particles. The monocyte is filled with particles (*L*) which also adhere to its plasma membrane. The Sézary cell shows no engulfed particles. Magnification ×3300.

FIG. 5. A Sézary cell in the center of an E-rosette. Magnification ×3300.

more than two cytoplasmic invaginations into the nucleus or two invaginations deeper than half the diameter of the nucleus were counted as Sézary cells. Such cells were not seen in the blood of normal subjects. The sections were contrasted with uranyl acetate (25) and lead citrate (26) and viewed with a Siemens Elmiskop I electron microscope.

RESULTS

The patients with Sézary syndrome had more than 40% ultrastructurally abnormal cells among purified lymphocyte fractions. The incidence of Sézary cells in the blood of patients with mycosis fungoides varied from 0 to 30%. The ultrastructure of Sézary cells (Fig. 1*a* and *b*) has been described by others (27, 28), who have stressed the convoluted nucleus and high glycogen content. However, it had not been noted heretofore that at high resolution the cytoplasm of most of the cells is seen to be replete with fibrils that measure about 75 Å in diameter and are of indeterminate length (Figs. 2 and 3). The structures may be straight, curved, or undulating and, at times, resemble those described in "monocytoid" cells found in amyloid-laden lymph nodes (29). None of the Sézary cells phagocytosed latex particles, which were readily ingested by monocytes present in the same specimen (Fig. 4). This observation contradicts the view that the Sézary cell is a type of histiocyte or monocyte, as suggested in the past (18). In contrast to the cells that circulate in CLL, Sézary cells carried no surface immunoglobulin or receptors for complement. Moreover, B-cells were present in low amounts (<4%) or absent from the blood of all patients (normal range 5–10%). Normal or high levels of T-lymphocytes, as measured by E-rosettes, were present in each instance. Although, on light microscopy, the leukocyte in the center of a tight rosette is not easily identifiable, electron microscopy clearly established the presence of Sézary cells in most, though not all, E-rosettes (Figs. 5 and 6). No Sézary cells were identified within EAC-rosettes. In general, the percentage of T-lymphocytes was greater than the number of Sézary cells in the same specimen, but not all Sézary cells were able to form E-rosettes (Fig. 7).

Of 11 specimens cultured with PHA, eight showed an excellent response, two had a poor response, and one failed to respond. In one subject, treated with chemotherapy, the percentage of Sézary cells decreased from 70% to 2%, but the number of B-cells remained low and there was no longer any response to PHA.

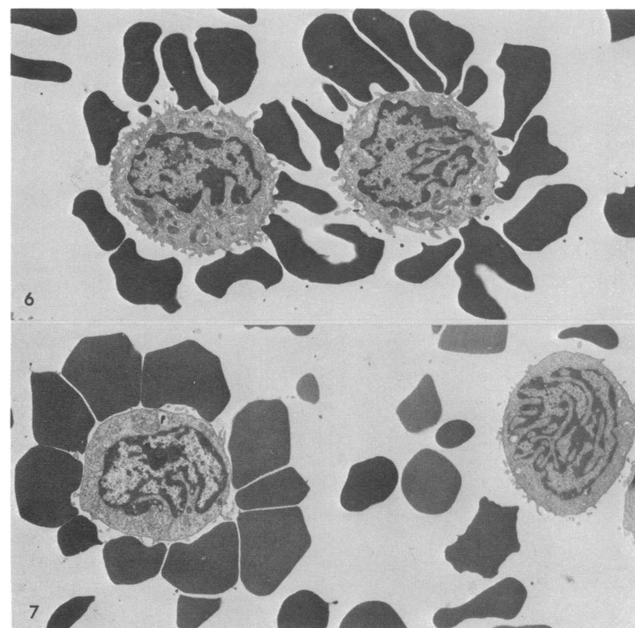

FIG. 6. Two lymphocytes that formed E-rosettes. The one on the *right* is a definite Sézary cell, as judged by the shape of its nucleus. The cell on the *left* cannot be identified in this particular plane of section and could be a normal T-cell. Magnification ×3300.

FIG. 7. Examples in which a typical Sézary cell (*right*) has failed to form an E-rosette, whereas the cell on the *left* has formed a tight rosette. Magnification ×2750.

DISCUSSION

The observations reported here support the view that the so-called Sézary cell is an abnormal variant of a T-lymphocyte. The cell lacks surface immunoglobulin as well as receptors for immunoglobulin and complement; it is nonadherent and nonphagocytic. By current criteria, these findings speak against the possibility that the cell represents a "reticulo-histiocyte," a monocyte, or B-lymphocyte. It is puzzling why, despite normal levels of bone-marrow plasma cells and serum immunoglobulin, all patients had extremely low numbers of circulating B-lymphocytes or none at all. Whether this represents only a relative deficiency or whether B-cells were replaced by neoplastic T-lymphocytes in lymphoid organs is not certain.

The evidence that the Sézary cells is indeed a T-lymphocyte is of necessity indirect. Several findings argue in favor of this view. (*1*) It has been demonstrated, with the aid of electronmicroscopic analysis, that Sézary cells occupy the center of E-rosettes (Figs. 5–7). To date, no other cells have been shown to form spontaneous rosettes with sheep erythrocytes. (*2*) The cells appear to be able to undergo blast transformation and DNA synthesis when stimulated with PHA. Though the latter may not be an exclusive feature of lymphocytes, we have shown in other studies that our lymphocyte preparations are virtually devoid of other leukocytes (20). (*3*) It has been claimed that "most, but not all Sézary cells" can be killed with antiserum cytotoxic for T-cells (30). If one accepts the premise that the Sézary cell is a

T-lymphocyte, the following features distinguish it from a normal T-cell: (*a*) variability in cell size, already commented on by others (31, 32), (*b*) the highly convoluted nucleus (Figs. 1–7), (*c*) a large amount of glycogen (28, 33), and (*d*) a prominent network of cytoplasmic fibrils (Figs. 2 and 3). The fibrils have not been commented on by others, but perusal of publications on the Sézary syndrome showed fibrillar structures in some illustrations (Fig. 7 in ref. 28 and Fig. 8 in ref. 34). The significance of such fibrils has eluded definition for some time. They have been referred to as tonofibrils and stress fibrils, and were found by one of us in large numbers in the reticuloendothelial and other "monocytoid" cells in the lymph nodes of two patients with amyloidosis (29). Moreover, they appear to occur in other neoplastic cells (35, 36), in rabbit peritoneal macrophages (37), as well as in occasional human thoracic duct lymphocytes (38). Although contact between fibrils and the nuclear membrane was not seen, the abundance of such fibrils in Sézary cells raises the question of their relationship to the deformity of the nucleus.

The malignancy of the Sézary cell has been clearly established by the finding of abnormal karyotypes in certain patients (32, 39). Though cytogenetic studies on our patients have not yet been extensive†, the small Sézary cells of C.S. were aneuploid, whereas the large cells of M.P., had a normal karyotype. Thus it remains to be seen whether classification into a large-cell and a small-cell variant, as pointed out by Lutzner (32), will prove to be useful. We have observed small cells with an average diameter of 10 μm as well as large cells with a diameter of about 20 μm in one of our specimens. The clinical argument whether or not the Sézary syndrome should be considered a separate disease entity from mycosis fungoides is not relevant to this discussion. It seems more cogent to ask whether similar cells, when found in other lymphoid tumors, particularly those involving the skin (40), also consist of T-lymphocytes. To date Sézary-like cells have been described in the biopsy specimen of a patient with cutaneous reticulum cell sarcoma and in two subjects with the benign condition, lichen planus (41). Tests for cell-surface markers had not been carried out in these cases. We hope that the recent introduction of a technique that may permit localization of T-cells in normal lymphoid organs (42), may be of help in the detection of other lymphoid neoplasms of possible T-cell origin.

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