Light-Microscopic Analysis of Sectioned Sézary Cells

An Accurate Alternative to Electron Microscopy

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The prognostic implications of circulating Sézary cells in mycosis fungoides (MF) are not known, and the significance of fluctuating Sézary cell counts in either MF or the Sézary syndrome has not been assessed. Such studies have been hampered by the inaccuracy of counts performed on routine blood smears and the unavailability of electron microscopy for routine purposes. The present studies conducted on the peripheral blood of 35 patients with either MF or the Sézary syndrome show that Sézary cell counts performed by light microscopy of sectioned Epon-embedded lymphocyte fractions are as accurate as those carried out at the ultrastructural level. In addition, the studies include preliminary observations concerning 20 patients whose Sézary cell counts were repeated over time intervals ranging from 3 months to over 5 years. The described method should facilitate the performance of blood and lymph node Sézary cell counts on a wider scale, which is a necessity if the significance of circulating Sézary cells is to be evaluated. (Am J Pathol 1980, 99:243-252)

THE SÉZARY SYNDROME (SS) was first described in 1938 by Sézary and Bouvrain as a generalized exfoliative erythroderma with "monstrous" mononuclear cells in the peripheral blood.[1] Additional features of the disease may include hepatomegaly, splenomegaly, lymphadenopathy, pruritis, keratoderma, alopecia, and onychodystrophy. The dermatopathology is remarkable for the presence of large hyperchromatic lymphocytes with scant cytoplasm and deeply folded cerebriform nuclei. The same pathology is seen in mycosis fungoides (MF), a primary cutaneous lymphoma first described by Alibert in 1806.[2] Most investigators now agree that the Sézary syndrome represents the leukemic variant of mycosis fungoides and that both conditions are attributable to a malignant transformation of a subset of T lymphocytes.[3-6] Mycosis fungoides demonstrates a wide clinical spectrum and the duration of the disease is extremely variable. In fact, with the exception of the advanced stages of the disease manifested by tumors, ulcers, and lymph node involvement where the median survival is estimated to be less than 5 years,[7-10] the prognosis for an individual patient is impossible to predict. Therefore, it is also difficult to evaluate the effect of treatment or to decide when conservative

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topical management should be replaced by more aggressive systemic chemotherapy. As in other lymphomas, recent attempts at more precise staging including modern radiographic techniques have been of some help. In the case of MF the staging criteria have not yet included an enumeration of circulating Sézary cells. This is due in part to the notorious inaccuracy of Sézary cell counts carried out on routine blood smears and the time and effort involved in performing such studies on the ultrastructural level.

Encouraged by the suggestion that fluctuations of the circulating Sézary cell count may be directly related to clinical exacerbations and remissions,[11,12] we commenced a long-term study to assess whether the number of Sézary cells in the blood of patients with SS or MF has any prognostic significance. In the event that blood Sézary cell counts would prove useful and thus be warranted on a wider scale, we also investigated whether light-microscopic analysis of Epon-embedded specimens would be as accurate as counts carried out by electron microscopy. The data to be reported here show that there is an excellent correlation between blood Sézary cell counts determined by electron microscopy and those performed by light microscopy on Epon-embedded lymphocyte fractions. In addition, we record preliminary observations concerning 20 patients with varying stages of disease whose Sézary cell counts were repeated at time intervals ranging from 3 months to more than 5 years.

Materials and Methods

Patients

Thirty-five randomly selected patients with either mycosis fungoides (28) or with the Sézary syndrome (7) who ranged in age from 17 to 78 years were followed for 4 months to more than 5 years. In all cases, the diagnosis was confirmed by histopathologic studies. The stage of disease was ascertained by the diagnostic workup modified from the one recommended by the National Cancer Institute.[13] In brief, Stage I, the frequently misdiagnosed patch stage, consisted of a macular, poikilodermatous, slightly scaly eruption; Stage II was characterized by red, scaly plaques; Stage III featured nodular lesions or frank tumors; Stage IV involved lymph nodes; and Stage V signified extracutaneous and extranodular spread. These five stages were further classified according to the total area of skin affected, Group A representing less than 20% of cutaneous involvement, Group B greater than 20%. Thus, the generalized infiltrated plaques of the Sézary syndrome constituted Stage II B. Gallium scans were often substituted for lymphangiograms.

Preparation of Cells

Fifty milliliters of heparinized venous blood was initially obtained from each patient and at repeated intervals from 20 of these subjects. Within 1 hour of collection mononuclear cells were isolated by density gradient centrifugation through Ficoll-Hypaque, and the monocytes were eliminated with lymphocyte separator reagent (Technicon Inc., Tarrytown, NY) as described in detail elsewhere.[14] To verify that all monocytes had been removed, some specimens were incubated with 30λ latex particles (Dow Chemical Co., Midland, Mich) 0.3 in diameter for 15 minutes [14] before fixation. This insured the recogni-

tion of contaminating monocytes that, because of their lobulated nucleus, might be mistaken for Sézary cells in sections viewed by light microscopy. The specimens were fixed in 3% glutaraldehyde, dehydrated, and embedded in Epon, as is routine in this laboratory. Thick (1-μ) sections were stained with toluidine blue for light microscopy and adjacent thin sections were contrasted with uranyl acetate and lead citrate for electron microscopy. The criteria for the identification of Sézary cells on the electron-microscopic level have been published.[4,15] The features used for identification of the cells in thick sections were not different from those used by others in the interpretation of blood smears or skin biopsies.

A minimum of 500 cells were counted in each sample, care being taken that the sections represented different block faces. The specimens were coded. The electron-microscopic and light-microscopic counts were performed by different observers who had no knowledge of the results obtained by the other method.

The peripheral blood smears made from the same specimens were examined by a hematology technician trained in the recognition of Sézary cells. This person was informed that all specimens were obtained from patients with MF, and he was asked to record the percentage of Sézary cells among 100–200 lymphocytes.

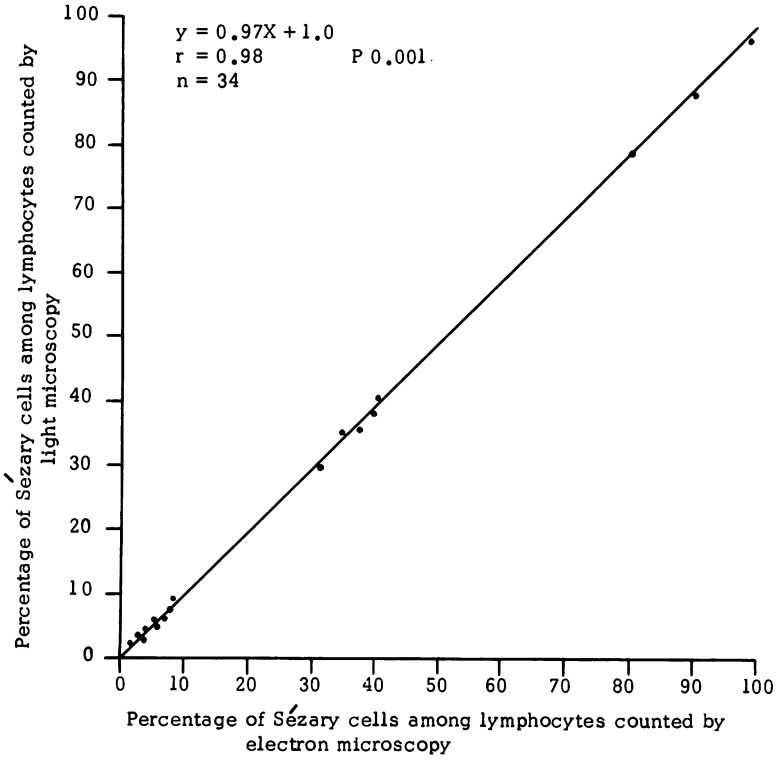
Results

An electron micrograph of a Sézary cell is shown in Figure 1. This cell was chosen to illustrate the narrow invaginations of the cytoplasm into the nucleus that could not possibly be resolved by light microscopy of smears containing unsectioned cells. It is likely that the contour of such a nucleus would appear round in Wright- or Giemsa-stained smears, and the cell would therefore not be recognized as a Sézary cell. On the other hand, light microscopy of toluidine-blue stained thick sections allowed for easy recognition of Sézary cells (Figure 2). Table 1 shows the excellent correlation between counts obtained on electron-microscopic examination of thin-sectioned and light-microscopic examination of thick-sectioned Epon-embedded material. The discrepancy is insignificant, as indicated by a correlation coefficient of 0.9 (Text-figure 1). The standard deviation obtained by counting sections of the same sample 10 times was ± 4.9.

On the other hand, there was no correlation between counts performed on blood smears and those obtained by the other two methods (Table 1). This confirmed our earlier impression that such counts are unreliable.

Table 2 records the clinical stage and Sézary cell counts obtained on 20 patients whose workup was repeated at variable time intervals.

Our preliminary observations are as follows: 1) Clinical progression or remission of MF as judged by the degree of skin involvement is not necessarily accompanied by a rise or fall in circulating Sézary cells, independent of whether this count is high or low. 2) Patients with progressive tumor-stage MF leading to early demise may retain persistently low blood Sézary counts during the entire course of their disease (eg, Patients 4 and 5). 3) Some patients (eg, 1, 6, 7, 8, and 9) with early limited MF showed



TEXT-FIGURE 1—Correlation of Sézary cell counts obtained by light microscopy (ordinate) and electron microscopy (abscissa).

low, nonfluctuating Sézary cell counts. Note: An increase or decrease of 5% in the Sézary cell count is not significant. 4) Clinical improvement in some patients (eg, 10, 11, 14) was associated with decreased Sézary cell counts, and an increased Sézary cell count was seen in 4 patients who deteriorated clinically (eg, 15, 16, and 17). 5) A high Sézary cell count, ie, over 20%, is not necessarily rapidly fatal, as evidenced by the three patients (eg, 14, 19, and 20) with initial Sézary cell counts over 75% who have been leading reasonably normal lives for the past 5 years.

Discussion

The major aim of this study was to examine whether the accuracy of peripheral blood Sézary cell counts carried out by light microscopy of sectioned Epon-embedded lymphocyte fractions would be comparable to counts performed on the ultrastructural level. As seen in Table 1, this correlation did not only prove excellent but also confirmed our previous impression that ordinary blood smears are unreliable for this purpose. How-

Table 1—Percentage of Sézary Cells Among Lymphocytes

Patient (initials)	Electron microscopy	Light microscopy (sections)	Light microscopy (smears)
L.S.	30.7	26.0	22.0
H.F.	59.6	54.8	21.0
B.H.	57.4	55.0	
L.M.	11.1	16.8	21.8
J.M.	2.2	7.3	16.0
S.S.	18.3	21.3	25.0
P.J.	2.8	6.2	11.0
J.R.	10.0	8.9	
H.R.	1.7	6.1	18.0
J.M.	13.4	7.8	12.0
E.C.	6.4	4.1	25.0
W.R.	13.1	9.2	19.0
P.M.	22.9	17.1	13.0
R.H.	18.3	15.0	18.0
F.T.	2.2	4.3	
P.P.	1.9	3.0	
L.B.	71.8	79.6	
L.D.	77.2	80.5	67.0
R.Y.	6.4	7.6	38.0
P.W.	44.0	37.5	17.0
M.M.	27.7	31.6	26.0
S.C.	6.4	5.7	10.0
R.A.	4.8	4.2	20.0
R.R.	6.2	9.2	18.0
W.R.	98.0	94.0	98.0
W.R.	97.2	89.0	90.0
M.R.	3.1	3.3	
M.D.	43.0	39.7	24.0
O.E.	5.4	3.4	19.0
A.W.	45.0	41.0	21.0
L.D.	12.7	6.5	14.0
C.A.	41.3	36.0	17.0
P.P.	5.2	5.4	17.0
R.B.	6.9	3.5	17.0
L.F.	16.0	14.1	14.0

Percentage of Sézary cells among isolated peripheral blood lymphocytes assessed by electron microscopy, light microscopy of Epon-embedded sectioned cells and routine blood smears.

ever, one proviso seems in order: The lymphocyte preparations should not be contaminated with monocytes or other granulocytes. Although it is not difficult to distinguish monocytes from Sézary cells by electron microscopy, it is not always feasible by light microscopy, even with the help of a 100× oil immersion objective. Since monocytes are avidly phagocytic and Sézary cells are not,[4] incubation of the cell suspension with latex particles before fixation will facilitate recognition of the former cells. This maneuver is recommended until the isolation of lymphocytes has become a routine procedure. On the other hand, plastic embedding methods with to-

Table 2—Clinical Stage and Sézary Cell Counts of 20 Patients Whose Blood Sézary Cells Were Quantitated at Various Time Intervals

Patients	Initial stage	Initial Sézary cell count	Treatment	Time interval (years)	Follow-up stage	Follow-up Sézary cell count (%)	Clinical* stage	Sézary† cell count
1 R.Y.	II A	6.4	HN2 topical steroids	2.0	II A	6.0	–	–
2 S.B.	III B	4.0	HN2 topical steroids Electron beam	2.0	II B	2.4	↑	↓
3 M.C.	III A	4.0	X ray	2.0	0	5.9	↑	↑
4 C.G.	III A	7.0	HN2 PUVA chemotherapy	2.0	V	4.3	↓	↓
5 J.Sh.	III B	—	HN2 X ray	0.6	V	5.0	↓	
6 L.D.	I A	9.0	Topical steroids	3.0	0	6.5	↑	↓
7 S.C.	I A	6.3	Topical steroids	3.0	I A	6.4	–	–
8 M.W.	II A	3.7	Topical steroids HN2	3.0	I A	6.9	↑	↑
9 O.E.	II A	2.6	Topical steroids HN2	3.0	0	5.4	↑	↑
10 F.L.	IV	22.0	Topical steroids HN2	2.0	III	8.1	↑	↓
11 P.P.	V	21.0	HN2 topical steroids Chemotherapy	6.0	II A	1.9	↑	↓
12 F.B.	II B	20.0	HN2	2.0	II B	7.0	–	↓
13 J.M.	II B	15.7	HN2 topical steroids Electron beam	3.0	II B	2.2	–	↓
14 H.F.	II B	89.0	Telo X ray Topical steroids	2.0	II A	55.4	↑	↓
15 M.D.	II B	34.0	HN2 topical steroids Electron beam	0.6	III	43.0	↓	↑
16 A.F.	II B	9.0	HN2 topical steroids	2.0	II B+	35.0	↓	↑
17 L.F.	II A	8.9	HN2 topical steroids	2.0	II B	25.0	↓	↑
18 J.C.	II B	17.7	HN2 chemotherapy Topical steroids X ray	5.0	II B	25.9	–	↑
19 C.A.	II B	75.0	HN2 topical steroids PUVA	2.0	II B	82.1	–	↑
20 W.R.	II B	95.0	Chemotherapy Topical steroids	2.0	II B	97.7	–	–

* Clinical stage: improved ↑; deteriorated ↓; stable –.
† Sézary cell count: increased ↑; decreased ↓; stable –.

luidine blue staining are common practice in most pathology laboratories, and therefore it should not require much effort to train technical personnel to identify the cells in specimens prepared from blood or lymphoid tissue.

If has often been questioned whether cells with the morphologic features of Sézary cells are present in the blood of healthy subjects or patients with cutaneous disease other than mycosis fungoides.[16]

In a large study recently published by Guccion et al [17] an attempt was even made to subclassify the cells into those with moderately convoluted nuclei and those with markedly convoluted nuclei when studied by electron microscopy. Although the latter configuration appears to carry more significance, the authors concluded that only clustering of such cells in biopsy material may be of importance. Guccion et al did not find Sézary cells in the peripheral blood of 2 patients with benign cutaneous disease. In our laboratory, lymphocytes with convoluted nuclei are occasionally found in the blood of healthy individuals, but only in very small numbers. In order not to mute the role of the Sézary cell as a specific marker for MF or SS, we consider a Sézary cell count of less than 5% of the total lymphocyte count insignificant. This practice is analogous to the custom adopted by hematologists in considering a critical number of bone marrow plasma cells, ie, 20% or over, as significant in the diagnosis of multiple myeloma.

In regard to fluctuations in Sézary cell counts during the course of the disease, with or without chemotherapy, our data are still too preliminary to permit any firm conclusions. Previous studies attempting to correlate circulating Sézary cells with improvement or deterioration of disease were based on examination of peripheral blood smears,[10,12] a method we found to be extremely inaccurate. The simplicity and reproducibility of Sézary cell counts that can be obtained by plastic embedded sections of purified lymphocytes ought to encourage many investigators to collect more meaningful data on a wider scale in the future.

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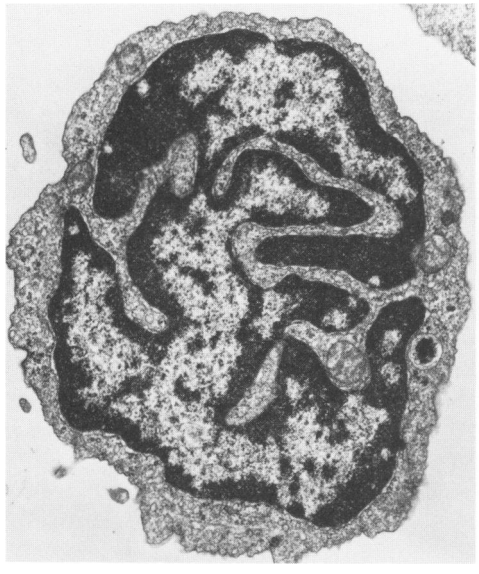

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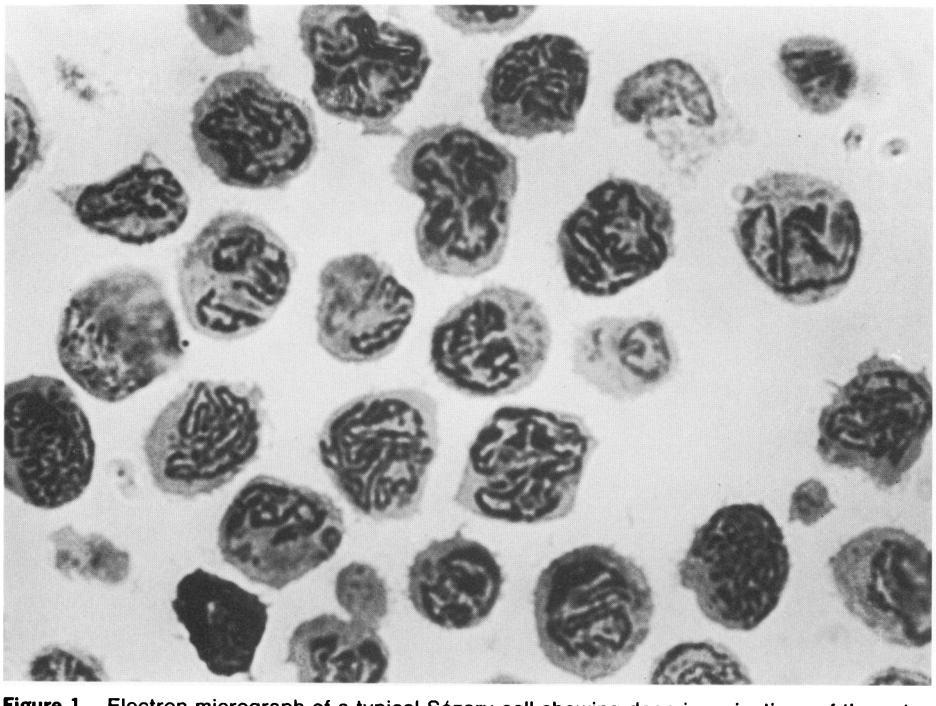

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Figure 1—Electron micrograph of a typical Sézary cell showing deep invaginations of the cytoplasm into the nucleus. The invaginations are too narrow for resolution by light microscopy. Therefore, it is likely that the nucleus of this cell would appear to possess a smooth, round contour when viewed by light microscopy. (×11,000) **Figure 2**—Section of Epon-embedded Sézary cells, stained with toluidine blue, examined by conventional light microscopy, shows the convoluted nuclei to good advantage. (×3000)

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