Ultrastructure and Cell Marker Studies in Lymphoproliferative Disorders

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Surface marker studies have been shown to provide new objective criteria which help to define normal and leukemic cells. Methods for recognizing different subpopulations of lymphocytes are in sharp contrast to conventional morphological techniques, e.g. Romanowsky stained films and paraffin sections, which not only do not allow such distinction to be made, but fail sometimes even to demonstrate the lymphoid or myeloid nature of a neoplastic process. Transmission electron microscopy (TEM) increases the precision of morphological analysis of cell types by demonstrating in greater detail, and with less artefacts, such cell characteristics as nuclear chromatin condensation, nuclear shape, details of cytoplasmic organelles, inclusions, etc. The combination of TEM and surface marker studies is bound, therefore, to increase our power of identification and characterisation of the cells involved in lymphoproliferative disorders.

Materials and Methods

TEM and surface markers were performed in peripheral blood and/or bone-marrow samples from acute and chronic leukemias; lymph node biopsy specimens from non-Hodgkin's lymphomas were also tested. The disorders studied were: chronic lymphocytic leukemia (CLL), leukemic phase of diffuse poorly differentiated lymphocytic lymphomas (PDLL) and follicular lymphoma (FL), hairy-cell leukemia (HCL), prolymphocytic leukemia (PLL), acute lymphoblastic leukemia (ALL), and Sezary's syndrome. The B- and T-cell markers, namely, surface membrane immunoglobulins (SmIg) and rosetting tests with mouse and sheep RBC cells [2,4] were investigated in 194 cases. Cases of ALL were also investigated with an anti-ALL serum to detect the "common-ALL" antigen (Dr. M.F. Greaves, Imperial Cancer Research Fund) [5], and for the enzyme terminal transferase (TdT) (Professor A.V. Hoffbrand and K. Ganeshaguru, Royal Free Hospital) [7]. TEM was performed in 80 cases. In several instances cytochemical techniques for acid phosphatase, myeloperoxidase and immunoperoxidase were also studied at TEM level. For TEM the specimens were fixed in 3% glutaraldehyde and processed according to standard techniques. The material was embedded in Araldite and ultrathin sections were viewed with an AE1 6B electron microscope.

Results

The terms "differentiated", "poorly differentiated" and "undifferentiated" used to define certain conditions, will refer only to the morphological appearances.

Differentiated B-cell Disorders

These include B-cell leukemias: CLL, PLL, HCL and FL. In all the cases one or both of the B-cell markers used were positive.

Morphology

The predominant cell seen in *B-CLL* is a small lymphocyte with scanty cytoplasm, heavily condensed nuclear chromatin and inconspicuous Golgi apparatus (Fig. 1). Prolymphocytes predominate in *B-PLL;* they have a prominent nucleolus in the presence of peripheral chromatin condensation (Fig. 2). A spectrum of cells, depending on the degree of chromatin condensation and the shape of the nucleus can be seen in *B-PLL. FL* is characterized by cells with cleaved (notched) nuclei with variable degrees of chromatin condensation. Small cleaved cells are seen particularly in the peripheral blood in the cases associated with high WBC (Fig. 3); large cleaved cells with less chromatin condensation were predominant in the lymph nodes of the same cases.

Surface Markers

According to the density of SmIg, reflected in the intensity of the immunofluorescence reaction with polyvalent and monovalent antisera, cases were defined as having negative, weak, moderate or strong fluorescence. In B-CLL, 80% of cases had a weak to moderate reaction, and in 17% of cases SmIg were undetected; in contrast, 90% of B-PLL cases had a strong reaction. In FL, PDLL, and HCL, about half the cases had weak to moderate intensity and the rest had a strong reaction.

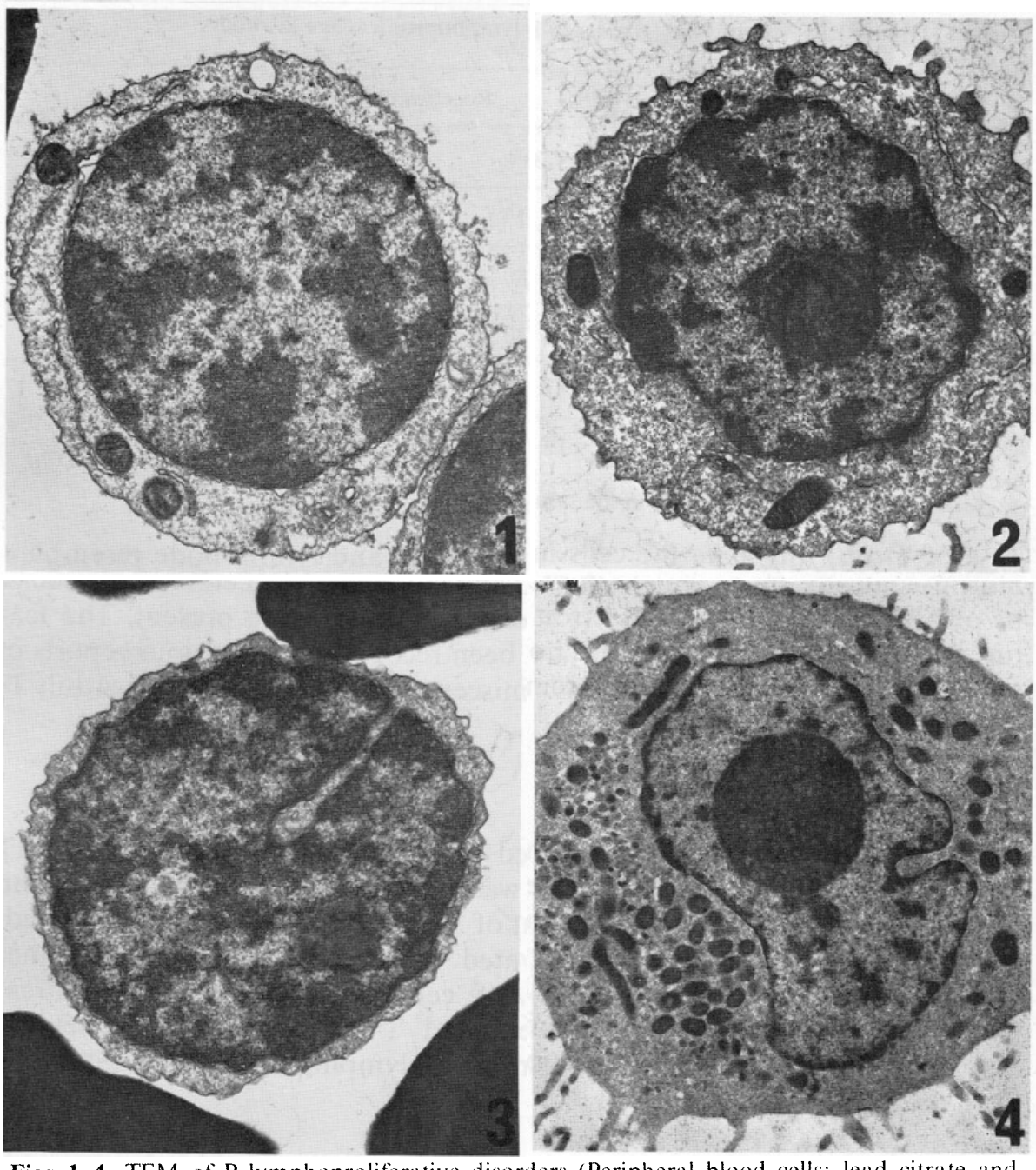
Results with the mouse RBC rosette test (neuraminidase-treated lymphocytes) are shown in Table 1. More than 50% rosettes were observed in the majority of B-CLL cases. In contrast, this was rarely the case in the other conditions where the common finding (73 to 87% of cases) was a low binding (<30% rosettes) of mouse RBC; only 1% of CLL cases showed a similar finding.

Differentiated T-cell Disorders

These include T-CLL (3 cases), Sezary syndrome (4 cases) and a case of T-cell lymphoma.

Morphology

A common feature of the cells of these cases was the irregular nuclear outline, a prominent Golgi zone, and the presence (in variable proportion) of



Figs. 1–4. TEM of B-lymphoproliferative disorders (Peripheral blood cells: lead citrate and uranyl acetate stain)
1. Typical lymphocyte in CLL (× 13000)
2. Prolymphocyte in PLL: prominent nucleolus and peripheral chromatin condensation (× 13000)
3. Small cleaved lymphocyte in FL (× 13000)
4. B-ALL. lymphosarcoma type. Larger size and no peripheral chromatin condensation main difference from PLL (× 11000)

electron dense granules in the cytoplasm, which showed acid phosphatase reactivity. The granules seen at E/M correspond to the azurophil granules seen at light microscopy [1]. In *T-CLL* the nucleus was frequently, but not always, irregular; rarely it resembled the Sezary cell (Fig. 5). In the Sezary cells the main feature was the very convoluted (cerebriform) nucleus (Fig. 6) with condensed chromatin with or without a prominent nucleolus. In the

Table 1. Mouse RBC rosettes in 166 cases of B-lymphoproliferative disorders

Disease	No. of cases	Rosettes in peripheral blood samples[a]		
		<30%	30–49%	≥50%
CLL	100	1 (1%)	21 (21%)	78 (78%)
PLL	20	16 (80%)	4 (20%)	—
FL	11	8 (73%)	1 (9%)	2 (18%)
PDLL & B-ALL	15	13 (87%)	—	2 (13%)
HCL	20	10 (59%)	7 (41%)	—

[a] More than 50% leukaemic cells in the samples tested

case of *T-cell lymphoma* the cells in the blood and lymph node resembled morphologically those seen in T-CLL and some had the cerebriform nucleus of Sezary cells (Fig. 7); electron dense granules were present. The features of this condition, which has not been recognized in previous reports of non-Hodgkin's lymphomas, are reminiscent of those described as Adult T-cell leukemia in Japan [10].

Surface Markers

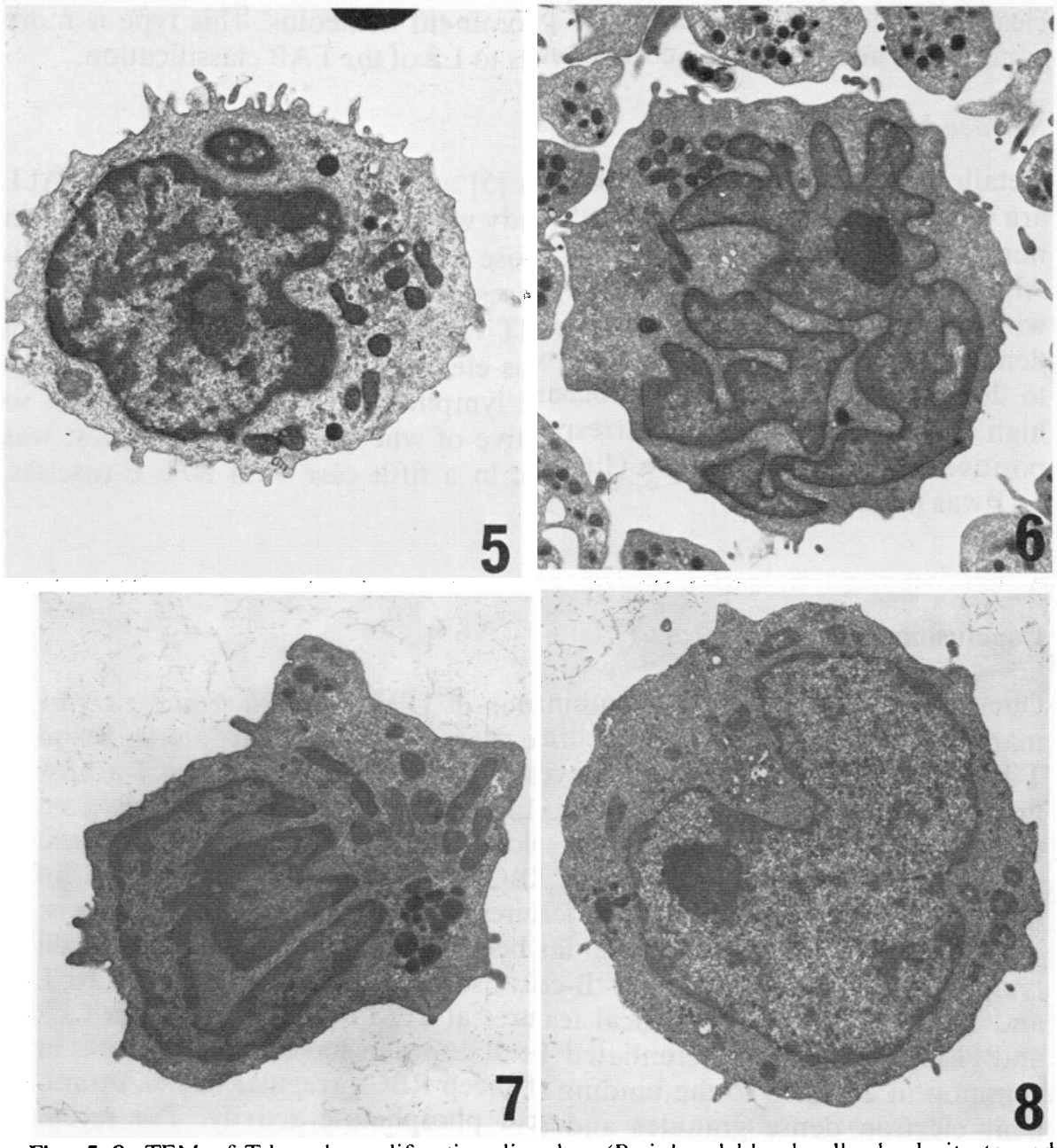
In all cases the leukemic cells formed rosettes with sheep RBC cells. TdT estimations showed that the enzyme was undetectable in the 3 T-CLL's, the case of T-cell lymphoma and in 2 out of 3 cases of Sézary syndrome tested. One of the last-mentioned had elevated values: 22,4 U/10^8 cells (normal values in bone marrow up to 1,5 U/10^8 cells) [7]. These findings contrast with those seen in the less differentiated T-cell disorders (see below). Similar results with TdT estimations in T-lymphoproliferative disorders were recently reported by Penit et al. [8].

Poorly Differentiated or Undifferentiated Disorders

These include conditions characterised by the proliferation of blast cells (with little or no heterochromatin): ALL, PDLL and lymphoblastic lymphoma.

Morphology

B-ALL cells have either features resembling those seen in Burkitt's lymphoma, namely, lack of nuclear maturation with fat globules and polyribosomes in the cytoplasm (L3 in the FAB classification), or those of PDLL with an irregular nuclear outline and a very large nucleolus (lymphosarcoma type) (Fig. 4). The latter cells are distinct from PLL (Fig. 2) because they are larger and have no nuclear chromatin condensation. In *T-ALL* and lymphoblastic lymphoma the nucleus is frequently irregular or convoluted with a



Figs. 5–8. TEM of T-lymphoproliferative disorders (Peripheral blood cells: lead citrate and uranyl acetate stain)

5. Lymphocyte with prominent nucleolus and electron dense granules in the cytoplasm in T-CLL (× 10000)
6. Typical Sézary cell with a prominent nucleolus (× 10000)
7. T-cell lymphoma cell with features resembling Sézary cells (× 11000)
8. Blast cell in a case of T-ALL with indented nucleus and a prominent Golgi apparatus (× 11000)

prominent Golgi zone (Fig. 8), where the acid phosphatase reaction is often localized. *Non-B, non-T ALL* includes two types of cells: 1. Small cells with a round or indented nucleus and inconspicuous nucleolus, some peripheral chromatin condensation and clumps of heterochromatin inside the nucleus. This type is seen more often in children and corresponds to L1 of the FAB classification. 2. Large blasts with more abundant cytoplasm, irregular nu-

clear outline, open chromatin and prominent nucleolus. This type is more common in adult ALL and corresponds to L2 of the FAB classification.

Surface Markers

Detailed accounts of surface markers [5] and TdT estimations [7] in ALL are given elsewhere. In the present study we observed high values of TdT in non-B, non-T ALL, particularly in those cases positive with Greaves anti-ALL serum [5,7]. The cells from 5 cases of adult ALL that were negative with the anti-ALL serum had high TdT values in 3 cases, and TdT was not demonstrable in 2. In T-ALL, TdT was elevated in the 3 cases tested (26,6 to 202 U/10^8 cells). In lymphoblastic lymphoma TdT values were not so high (4,5 to 15,4 U/10^8 cells) irrespective of whether the E-rosette test was positive (3 cases) or negative (1 case). In a fifth case with 80% E-rosettes, TdT was negative.

Conclusions

Our studies showed that the combination of TEM and surface and enzyme markers makes possible more accurate characterisation of neoplastic B and T lymphocytes than can be achieved with routine morphological assessment. B-CLL (the common form of CLL) can be distinguished clearly from other conditions with peripheral blood lymphocytosis by the typical morphology, the binding of mouse RBC rosettes and the weak pattern of SmIg. B-PLL also has well defined features by morphology and cell markers; its characteristic cells have not, so far, been found among the various B-cell lymphomas studied. The other B-cell disorders studied, PDLL, FL, HCL and B-ALL, have morphological features at TEM quite distinct from CLL and PLL. Cells in the differentiated T-cell disorders have several features in common in addition to the binding of sheep RBC: irregular nuclei, prominent electron dense granules and acid phosphatase activity. The recent report of Grossi et al. [6], describing the morphological features of the helper (T_M) and suppressor (T_G) T-lymphocyte subpopulations, is of great interest as the findings in the T_G cells resemble those seen in T-CLL. Two of our T-CLL cases were negative for the enzyme α-naphthyl acetate esterase, which appears to be characteristic of T_M cells [6], and one of them was positive for the Ia antigen, a finding also associated with T_G cells [9]. This, and the preliminary data of Uchiyama et al. [10] suggest that T-CLL may be a disorder of T-suppressor cells. TdT studies [7] help not only in distinguishing ALL from AML, but also between immature (poorly differentiated) and mature (differentiated) T-cell disorders. The combination of good morphology (TEM), surface markers, including Greaves's anti-ALL serum [5], TdT [7] and the acid phosphatase cytochemical reaction [3] permits a better identification and subsequent classification of most leukemias and non-Hodgkin's malignant lymphomas.

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