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Perspectives and Commentaries

Ultrastructural and Cytochemical Studies in Hematologic Malignancies

WALTER FEREMANS,* ROBERTE MENU* and C. H. J. BRACOPS†
**Laboratory of Pathology and Electron Microscopy and †Department of Internal Medicine, Université libre de Bruxelles*

In the past decade, the discovery of new ultrastructural cytochemical staining methods, the continuous expansion of monoclonal antibodies against specific membrane antigens and of ultrastructural probes such as calibrated gold particles [1], have generated considerable progress in the understanding of hematologic malignancies. These techniques may be very useful in establishing an accurate diagnosis in certain cases of acute undifferentiated leukemia (A.U.L.), in megakaryocytic leukemia and hairy cell leukemia.

Conventional ultrastructural study of bone marrow samples or buffy coated mononuclear cells isolated by Ficoll–Hypaque leads one to recognize several abnormal structures directly related to various hematologic malignancies. B-lymphocytes of chronic lymphocytic leukemia (B-CLL), of macroglobulinemia and myeloma cells sometimes contain intracytoplasmic crystals or intranuclear inclusions of monoclonal immunoglobulin related to the pathologic synthesis or secretion of this protein [2]. In μ or γ heavy chain diseases, special vacuolated plasma cells may be encountered [3,4]. The ribosome–lamella complexes described in about 50% of hairy cell leukemias [5] are not specific. Indeed, they are seen in chronic lymphocytic leukemia, lymphosarcoma cell leukemia, myeloma, macroglobulinemia, Sezary syndrome and monoblastic leukemia. Rhopheocytosis of ferritin particles is an early ultrastructural feature of erythroblastic differentiation. Various other cytoplasmic or nuclear inclusions have been described in acute leukemias with a strikingly high frequency in acute promyelocytic leukemia associated with disseminated intravascular coagulation [6].

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Address for reprints: Dr. W. Feremans, Laboratory of Pathology and Electron Microscopy, Université libre de Bruxelles, 808, route de Lennik – bât.C – B-1070 Bruxelles, Belgium.

The ultrastructural detection of myeloperoxidase (MPX) is of special interest in distinguishing lymphoblastic from myeloblastic cells in cases of undifferentiated acute leukemia and cases of chronic granulocytic leukemia (CGL) blast crisis. Indeed, ultrastructural techniques show weakly positive reactions in blasts whereas reactions are negative with light microscopy [7] (Fig. 1). MPX staining is localized within the Golgi area, short strands of endoplasmic reticulum, the nuclear membrane and a few small dense granules. In contrast to the ultrastructural pattern of myeloperoxidase, the platelet peroxidase (PPO) is exclusively present within the endoplasmic reticulum and the nuclear membrane of the megakaryocytic lineage. By transmission electron microscopy, this enzyme is detected only in platelets, megakaryocytes and promegakaryoblasts. Therefore this ultrastructural technique is critical in diagnosing acute megakaryoblastic leukemia [8] (Fig. 2). With subsequent maturation, megakaryocytic cells show a clear-cut membrane demarcation system, glycogen and a few scattered 'bull's eye' granules at the ultrastructural level. Peroxidase activity with similar localization as PPO is identified in hairy cells thereby demonstrating the expression of its hybrid lymphoid-monocytic phenotype [9].

Ultrastructural acid phosphatase detection is of special interest in the field of *T*-chronic lymphocytic (*T*-CLL), *T*-lymphoblastic (*T*-ALL) and hairy cell leukemias. Tartrate-resistant isozyme 5 (TRAP) is demonstrated with greater sensitivity by electron microscopy using sodium naphthol AS-BI phosphoric acid as substrate in cases of hairy-cell leukemia [10]. The stain is located in the perinuclear membrane, in the rough endoplasmic reticulum, in granules and in mitochondria. As indicated in a recent paper by Merchant *et al.* [11], a high proportion of mononuclear cells composing colonies grown from blood and spleen of patients

with hairy cell leukemia maintains the characteristic morphology and the light and ultrastructural cytochemical activity of hairy cells.

The non-specific lysosomal acid phosphatase demonstrated with β-glycerophosphate as substrate is seen in the Golgi area and in neighbouring lysosomal granules in *T*-ALL [12], while in *T*-CLL a focal paranuclear staining is seen primarily in granules. *B*-cell malignancies lack focal acid phosphatase positivity. Large granular lymphocytes (NK cells) also show a positive acid phosphatase staining in the parallel tubular arrays (PTA) with β-glycerophosphate (the Gomori method), suggesting the lysosomal origin of these structures [13].

Poorly differentiated monoblastic leukemias show very small granules negative for peroxidase but reacting for acid phosphatase at the ultrastructural level.

A third ultrastructurally localized enzyme is acid alpha-naphthyl-acetate esterase (ANAE) [14]. In myelomonocytic and monoblastic leukemia (M_4, M_5 of the FAB classification), staining is principally seen at the plasma membrane and neighbouring endocytic vesicles and is inhibited by sodium fluoride. In the helper variant of *T*-CLL, positive sodium fluoride resistant staining is encountered within Gall bodies (Fig. 3). *B*-cells and *T*-ALL are devoid of ANAE reactivity.

Ultrastructural demonstration of membrane 5'nucleotidase may be helpful in the diagnosis of borderline cases of common ALL and CGL blast crisis. Recently developed ultrastructural techniques using the combination of cytochemistry and immunogold probes are also very useful. A modification of the fixation procedure for PPO does not alter the reactivity of the membrane antigens for monoclonal antibodies directed against platelet glycoproteins. The French team of Breton–Gorius [15] suggests that with this technique PPO appears earlier in maturation than platelet glycoproteins. Other ultrastructural cytochemical techniques may also be combined with the use of the immunogold probes (Fig. 4).

In conclusion, the development of new ultrastructural cytochemical and immunocytochemical methods may bring new perspectives in the diagnosis of poorly differentiated leukemias, undifferentiated leukemias, or mixed blast cell proliferations.

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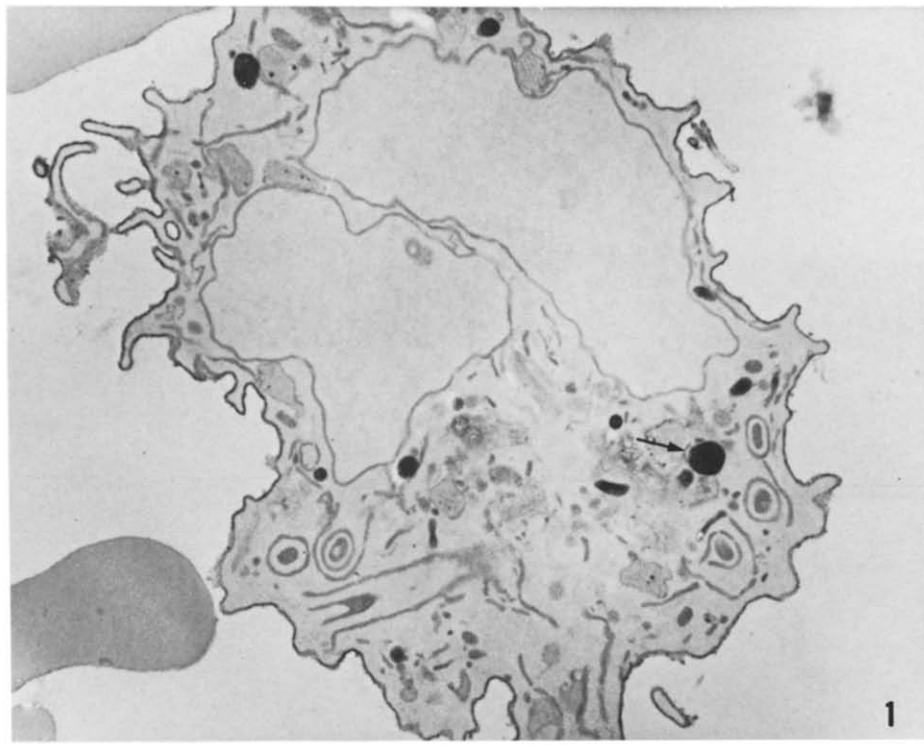


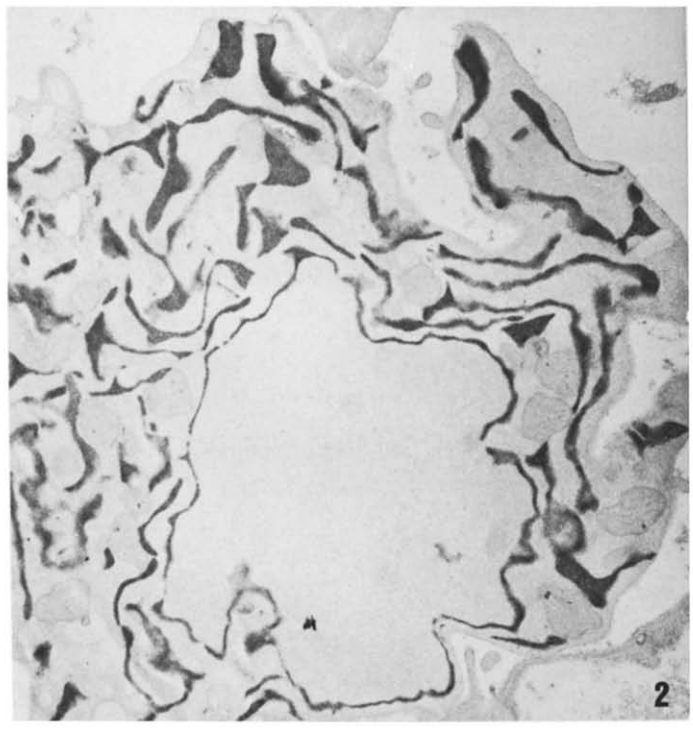
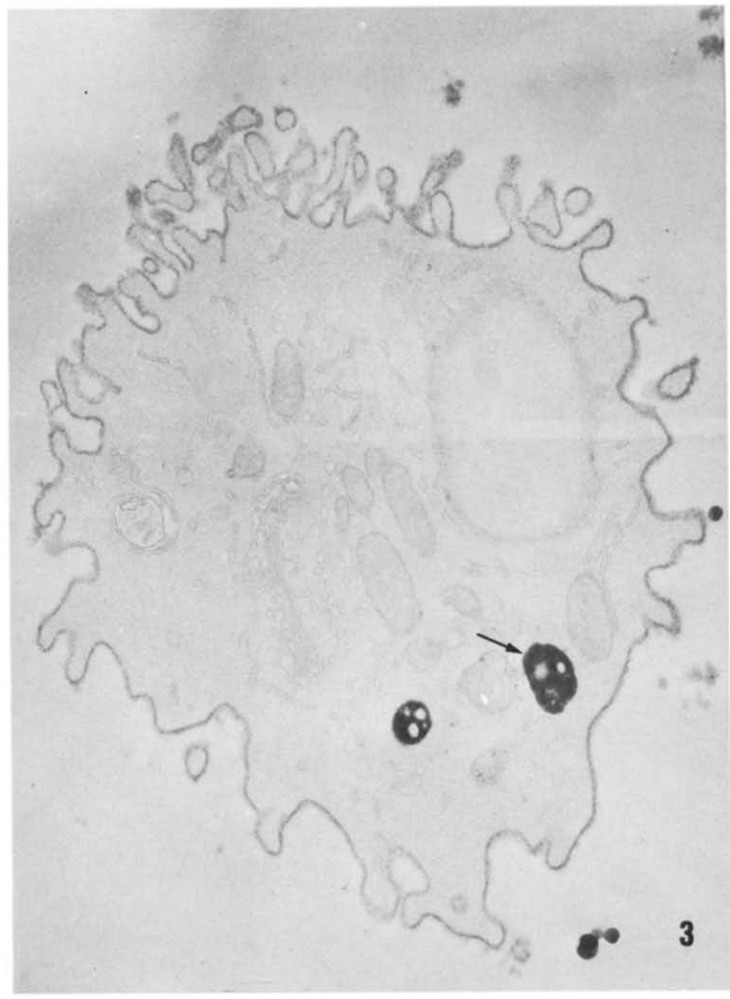


Fig. 1. *A promonocyte with myeloperoxidase positive granules* (→) (× 19,200).

Fig. 2. *A megakaryoblast stained for platelet peroxidase within perinuclear cisterna and rough endoplasmic reticulum* (× 19,200).



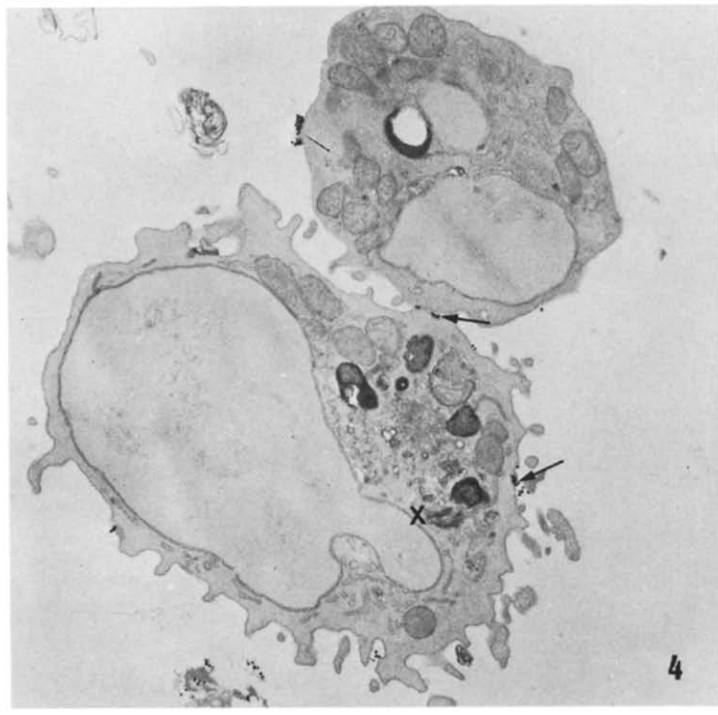


Fig. 3. *Alpha-naphthyl acetate esterase within the Gall bodies* (→) *of a helper* T-*cell* (× 24,000).

Fig. 4. *Acid phosphatase activity of the parallel tubular arrays* (X) *of a NK-cell stained at the cellular membrane by anti-Leu* 7 *with a immunogold technique* (→) (× 14,400).

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