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UNIQUE SCANNING ELECTRON MICROSCOPIC FEATURES OF HAIRY CELLS IN HAIRY-CELL LEUKEMIA.  
A REVIEW AND CURRENT STATUS

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Abstract
Past scanning electron microscopy (SEM) reports demonstrated cell surface undulations, 
ridges, folds, and ruffles to support the monocytic/histiocytic nature of hairy-cell 
leukemia (HCL) cells. On the other hand, SEM studies illustrating spikes, villi, and microvilli 
on the cell surfaces favored the lymphocytic nature of hairy cells (HCs). The evidence for the 
'hybrid' nature of the HCs has emerged from the demonstration of concurrent display of monocytic 
ruffles and lymphocytic (microvilli) surface features on each cell. Utilizing improved methods 
of sampling, fixation, and drying, the current status is that all HCs display microvilli and 
ruffles simultaneously. However, two distinct morphological types of HCs are acknowledged: cells 
showing ruffled areas next to clumps of microvilli (type A), and cells displaying microvilli 
terspersed among ruffles (type B). Each of the 
HCL cases reported in our studies had cells with either type A or type B surface features. 
Amazingly, these unique SEM features correlate well with the prevalent trend to classify HCs as 
malignant (villous) B-lymphocytes imitating (ruffled) monocytes in some functional respects.

Key Words: Hairy cell; leukemia; monocyte; 
lymphocyte; cell surface; ruffles; microvilli.

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Introduction
In 1923, a rare and unusual form of chronic 
leukemia was described by a German physician named 
Ewald. The outstanding features of the disease 
were a very large spleen and the presence of 
circulating mononuclear cells with many 
cytoplasmic projections. Ewald [1923] termed this 
leukemia 'leukemic reticuloendotheliosis' (LRE) in 
the belief that the characteristic cell involved 
in this disease was of 'reticulo-endothelial' 
origin. Since the late 1950's, this unique cell 
with its singular surface has invited much 
attention as the subject of many studies 
evaluating its functional, morphological and 
cytochemical characteristics. Thereafter, these 
cells have been variously designated as 
'neoplastic lymphoid reticular cells', 
'reticulolymphocytic cells', or 'hairy cells' 
[Gosselyn et al, 1956; Bouroncle et al, 1958; 
Mitus et al, 1961; James & Goodwin, 1963; Schrek & 
Donnelly, 1966; Rubin et al, 1969]. 
The incidence of hairy-cell leukemia (HCL), 
with its characteristic bone marrow involvement 
[Burke et al, 1974; Naeim & Smith, 1974], has 
been estimated at 2% of all the leukemias; this rate 
was underestimated in the past since many HCL 
cases were diagnosed as lymphocytic leukemia or 
lymphoma. The distinction between HCL and other 
hematological disorders is important because 
active treatment, such as chemotherapy, is 
required for lymphoproliferative diseases, whereas 
in the more chronic HCL a conservative approach to 
patient management is necessary [Catovsky, 1977; 
Golomb et al, 1983]. 
A great deal of controversy about the nature 
and origin of the hairy cells (HCs) prevailed 
through the late 1960's and mid 1970's. The cells 
displayed exaggerated cytoplasmic projections and 
differed morphologically from typical lymphocytes 
and monocytes, with some characteristics of each 
cell type seen by both light microscopy and 
transmission electron microscopy (TEM) [Yam et al, 
1971; 1972; Flandrin et al, 1973; Katayama et al, 
1973; Burke et al, 1974]. Various investigators 
presented evidence that these cells are either 
monocytes or histiocytes [Rappaport, 1966; Yam et 
al, 1968; Daniel & Flandrin, 1974; Jaffe et al, 
1974; Rozenszajn et al, 1976; Scheinberg et al, 
1976], while other workers contended that the HCs
On the other hand, many reports have indicated bacteria in vitro. Moreover, alpha-naphthylacetate esterase activity, which is present in monocytes and histiocytes [Skinnider, 1972; B-lymphocytes: Preud'Homme et al., 1972; Haak et al., 1974; Debuscher et al., 1975; Zidar et al., 1975; 1977; Deegan et al., 1976; Utsinger et al., 1977; T-lymphocytes: Cawley et al., 1978; Saxon et al., 1978].

Evidence favoring the monocytic/histiocytic nature of HCs has included the demonstration of the receptor for cytophilic antibody of monocytes on the HCs, the absence of the antigen-antibody complement receptor of B lymphocytes, and the ability of HCs to phagocytose latex particles and bacteria in vitro. Moreover, alpha-naphthylacetate esterase activity, which is present in monocytes and histiocytes [Yam et al., 1971; 1972; Katayama et al., 1972a; Janckila et al., 1978; Variakojis et al., 1980]. Tartrate-resistant enzyme activity has not been found in monocytes or histiocytes [Li et al., 1973]. Additional support for the lymphoid nature of these cells came from the demonstration of surface immunoglobulin as well as other immunological markers indicative of a B-cell origin of the HCs [Reiber et al., 1977; Burns & Lawley, 1979; Golomb et al., 1978].

HCs have also been studied by TEM since 1958 [Bouroncle et al., 1958] and by scanning electron microscopy (SEM) since 1971 [Trubowitz et al., 1971]. While the TEM findings have been fairly consistent and demonstrated the presence of a large ribosome-lamella complex in some HCs [Ghadially & Skinnider, 1972; Katayama et al., 1972b; Flandrin et al., 1973; Daniel & Flandrin, 1974; Burke et al., 1976; Katayama & Schneider, 1977], SEM findings have varied widely among investigators. In this regard we would refer the reader to a fascinating series of letters published in the "Lancet" in 1974 and 1975, where contradictory results on the typical SEM features of HCs were reported [Schnitzer & Hammack, 1974; Polliack et al., 1974; Roath & Newell, 1975; Catovsky et al., 1975; Schnitzer & Mead, 1975].

The early SEM studies, initially undertaken in the hope of solving the controversy concerning the nature and origin of HCs, revealed a surface covered with either microvilli and/or ruffles. Since, generally, microvilli are displayed by lymphocytes and ruffles by monocytes, the SEM results only added to the controversy. However, one clear benefit has resulted: these studies clearly establish a typical hairy cell surface morphology making possible the distinction between HCL and other types of leukemia on the basis of surface architectural features.

The present paper reviews the results of SEM studies of HCL cells. Possible reasons for the controversy on early SEM results are discussed in the light of the current status on the unique SEM features of HCs.

In a pioneering investigation, Trubowitz et al. [1971] demonstrated the anatomical and functional features of HCs from a patient with LRE (HCL) by time lapse cinematography, phytohemagglutinin stimulation, adherence to siliconized surfaces, cytochemical stains, and phase, transmission, and scanning electron microscopy. The hair-like processes that distinguished these cells in phase microscopy were seen as "spikes" and "broad membrane-like structures" after air drying of glutaraldehyde (GA) and osmium (Os) fixed cells. These morphological characteristics lead Trubowitz et al. [1971] to suggest that HCs are related to the monocytic or reticular cell systems. However, their histochemical and functional studies showed that the HCs also had a lymphocytic nature. It is worth noting that although the surface features of the air-dried cells were greatly damaged, these investigators were able to identify the equivalents of microvilli and ruffles which became the most characteristic SEM features of the HCs.

Another pioneer report in this area was that of Burns and Hoak [1973] who were the first to apply critical point drying (CPD) to the study of HCs under the SEM. Using comparative freeze-etching, scanning, and thin-section electron microscopy they studied the ultrastructure of abnormal mononuclear leukocytes from 3 patients with LRE (HCL). They described the abnormal cells as being covered with an extensive series of "pseudopods", "folds", "flaps", and other multishaped membrane "outpouchings" that obscured the underlying shape of the cell. Although their description was of ruffled-membranes (monocytic features) only, a review of their work shows that they actually demonstrated HCs covered by both ruffles and microvilli. The possibility that two different morphological types of HCs could exist was first considered by Polliack et al. [1974] and Golomb et al. [1975]. They studied HCs from the peripheral blood (PB) of 9 patients. In their studies, some cells displayed well-developed, broad-based ruffled membranes with a few microvilli frequently concentrated in a small area of the cell. Other HCs appeared almost as hybrids covered with equal proportions of well developed ruffles and finger-like microvilli. These reports suggested that the two types of cells, showing simultaneous features of both lymphocytes and monocytes, might in fact bear the markers of both B lymphocytes and monocytes, explaining why earlier reports had pointed to a B-derived cell origin, while others had favored a histiocytic/monocytic derivation of the HCs.

Dantchev and Belpomme [1977] have broadly used the SEM as a tool for the morphological analysis and classification of normal and pathological human mononuclear leukocytes. In their study, most of the cells from a case of B-type HCL, which had undergone CPD after GA and Os fixation, showed mixtures of ruffled and villous surfaces. However, cells from a case designated as "typical HCL" showed mixtures of smoothly-undulated and ruffled surfaces. Based on these results, they suggested that hairy cells...
Unique SEM features of hairy cells

Spleen cells from three patients with HCL were studied by Schnitzer & Hammack, under the SEM, in 1974. Examinations of cell and tissue specimens revealed that the HCs had the appearance and surface features of normal lymphocytes. Most of the cells displayed long slender villous processes but did not show the monocyte-like claimed that their results (i.e., the villous morphology of HCs) lent support to investigators advocating the B-lymphocyte nature for HCs. A year later, Schnitzer & Mead observed HCs from different organ sites (i.e., bone marrow, spleen) and surface characteristics similar to those described by Catovsky et al [1975]. The prepared cells had ridges and ruffles, resembling monocytes and histiocytes. Other cells had both villous processes and ruffles, giving a 'hybrid' appearance of both B lymphocytes and monocytes, as reported by Roath & Newell [1975].

SEM Studies Supporting the 'Hybrid' Nature of HCs

Nearly half of the cells observed by Roath & Newell [1975] in two HCL cases showed the spectrum of villous morphology consistent with that found in normal PB lymphocyte populations. These cells' surface characteristics were similar to those described by Schnitzer & Hammack [1974]. In both patients, a majority of cells bore the monocyte/lymphocyte surface features as described by Polliack et al [1974]. The 'hybrid' cells found in these studies had few well developed ruffled membranes, some ridge-like projections, and many stubby microvilli on their surfaces. Roath & Newell [1975] suggested that the morphological hybrid cell characteristic of the hairy cell was consistent with its monocyte-like phagocytic capability as well as its B-lymphocyte surface-marker activity.

A year later, Schnitzer & Mead observed HCs from the PB of two HCL patients and found cells with surface characteristics similar to those described by Catovsky et al [1975]. They found villi on cells from both cases, although some cells were similar to those described by Polliack et al [1974], displaying surface ruffles. A review of this study shows it did not exclude the possibility that the villous cells were actually a population of normal B-type lymphocytes which were present in the spleens of the patients.

Another interpretation of normal lymphocytes as HCL cells was probably done by Roath & Newell [1975] who studied the PB from two patients with HCL. They found villi on cells from both cases, although some cells were similar to those described by Polliack et al [1974], displaying surface ruffles. A review of this study shows it did not exclude the possibility that the villous cells were actually a population of normal lymphocytes which were present in the PB of the patients.

The last report in the literature describing all-villous HCs came from a study by Catovsky et al [1975] of spleen and PB cells from three HCL patients. These investigators claimed that their preparations of spleen HCs contained almost identical to those studied by Schnitzer & Hammack [1974], with surfaces thickly covered by cytoplasmic projections or villi of variable length. The possibility that normal B lymphocytes, present in the spleen, were responsible for the findings in the SEM was negated. In the same report, PB HCs were described as possessing thin processes (microvilli) and broad-based ruffled membranes resembling those seen in monocytes.

To explain the difference in the surface features of spleen as opposed to PB HCs, it was suggested that different environments might produce variant surface features, e.g., "packing" in an organ could result in interdigitating villi.
Current Status

Recent functional, ultrastructural, immunological, cytochemical, and cytogenetic studies have led to the current classification of HCL as a lymphocytic disorder involving malignant lymphocytes. Developmentally, these cells are placed late in the B-cell ontogeny, although some monocyte-like features are displayed [Catovsky et al, 1977; Reiber et al, 1977; Golomb, 1978; Golomb et al, 1984; Burns & Lawley, 1979; Jansen et al, 1979a,b; 1982; Yanovich et al, 1979; Golomb et al, 1980a,b; 1982; Rosner & Golomb, 1982].

Based on the surface features of cells derived from 15 different HCL patients and collected by the aspiration-filtration technique, Hamilton and co-workers [1984] identified three types of hairy cells: type A - cells showing large ruffles and areas of clumped microvilli, sometimes forming distinctive bipolar cells; type B - cells having predominantly microvilli interspersed between ruffles, and type C - cells displaying only ruffled surfaces. However, only single samples from two patients showed the type C subclass, and they were fixed only after being filtered onto silver membranes. The predisposition of microvilli to diminish from the surface of T lymphocytes collected by this technique has been previously demonstrated [Alexander et al, 1976]. Therefore, the cells classified as type C could easily belong to one of the two other subclasses, more likely to the second one.

In a recent study, the surface features of HCs obtained from 18 patients with HCL were reevaluated [Gamliel et al, 1985]. Spleen and PB cells were prepared for SEM by both conservative (i.e., CPD of samples fixed with either GA or GA and Os) and reformatory procedures (i.e., AD or CPD of samples treated with GA, tannic acid, guanidine-HCl, and Os). Generally, only two types of HCs were identified: (i) cells displaying areas of ruffles along side areas of clustered microvilli (Fig. 1a) and (ii) cells showing microvilli scattered among ruffles (Fig. 2a). PB HCs showed the same features as those isolated from the spleens involved with HCL (Figs. 1b, 2b), and consistently exhibited both microvilli and ruffles.

These results confirm the hypothesis that the HC is a unique entity in the SEM gallery of normal and leukemic lymphocytes [Golomb & Reese, 1976; Dantchev & Belpomme, 1977; Polllack, 1977], contrasting the reports on cases with all-villous or all-ruffled types of HCs [Schnitzer & Hammare, 1974; Catovsky et al, 1975; Roath & Newell, 1975; Hamilton et al, 1984]. The necessity of an accurate picture of the hairy cell membrane is most appreciated through current studies on the effect of in-vitro and in-vivo interferon treatment on the surface morphology of HCs [Gamliel et al, unpublished data]. In these SEM studies, consistent and reproducible surface characteristics are a prerequisite for obtaining reliable information that could shed light on the mechanism by which HCs are so efficiently eliminated from the blood of HCL patients treated with interferon [Quesada et al, 1984].

Finally, it is somewhat discouraging to note that none of the clinical and multidisciplinary studies done on HCs had provided evidence to support the above described two different SEM types of hairy cells [Hamilton et al, 1984; Jansen et al, 1984; Golomb et al, 1985]. However, it seems most likely that this topic will be clarified only by the harness of highly-sensitive immuno-ultrastructural methodologies to correlate between the immunological profiles and the morphological features of HCs.

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References


Dantchev D, Belpomme D (1977). Critical study of the mononuclear leukocyte morphology based on scanning electron microscopy in normal subjects and in patients with lymphoid or monocytoid
Unique SEM features of hairy cells

Figure 1: Type-A "hairy-cell leukemia" cells.
- Two PB HCs (top) showing distinct clusters of microvilli and ruffled membranes.
- Spleen HCs (from the same case shown in 1a) displaying numerous broad-based ruffles and clustered microvilli.

Figure 2: Type-B "hairy-cell leukemia" cells.
- PB HCs showing many small ruffles and evenly distributed microvilli.
- Spleen HC (from the same case shown in 2a) displaying microvilli intermixed with small ruffles.

proliferative disorders: Comparison with the T, B or null cell membrane phenotypes. Biomedicine 26, 202-222.


Unique SEM features of hairy cells


Discussion with Reviewers

D. Catovsky: Have you seen the observation of two types of HC been quantitated?
Authors: Yes. In our study of 18 patients 12 cases were classified as type A, and 6 cases as type B. In each of these cases more than 90% of the hairy cells were of the same type.

D. Catovsky: Can you state whether a particular HC type predominate or is seen exclusively in particular patients, both in peripheral blood and spleen or some patients have mixture of cells?
Authors: In all our studied HCL cases only one cell type predominated and none showed both types.

D. Catovsky: Have you studied systematically by SEM other cell types, CLL, PLL, etc., and have you not noticed more than one cell type by SEM?
Authors: We have studied more than 200 cases of leukemia under the SEM [Pollack et al, 1984. In: "Human Leukemias" (ed. A. Pollack), PD 405-418, Martinus-Nijhoff Publishing, Boston, MA]. In general, each subclass of leukemia cells had a characteristic surface morphology, and none showed more than one well-defined type of SEM features comparable to HCL (i.e., >90% of one type in case A and >90% of another type in case B).

D. Catovsky: Do you find useful or scientifically accurate to use the label 'monocyte' if a cell has ruffles and 'lymphocyte' if it has microvilli?
Authors: Yes, if we are dealing with untreated peripheral blood leukocytes showing only one type of surface microprojections. Using various comparative techniques including immuno-SEM we have never seen lymphocytes showing only ruffles or monocytes showing microvilli [Gamliel et al, 1983. J Clin Immunol 3: 399-407. Gamliel H, 1985. Scanning Electron Microsc 1985;IV: 1649-64].

G.B. Schneider: The surface features of normal lymphocytes differ depending upon the procedure used for drying, i.e., critical point drying vs. freeze drying (Billings-Gagliardi et al, Am J Anat 152: 383, 1977; Schneider et al, Scanning Electron Microsc 1978;II:77). Have you examined hairy cells prepared by freeze drying or are you aware of any such studies?
Authors: No, we did not prepare HCs by freeze drying and we are not aware of any such studies.

Reviewer III: The authors should indicate whether the presented figures illustrate air dried or critical point dried cells. They should also clarify on what ground they seem to identify all the cells observed here with hairy cells. Was any new cell separation procedure applied, and if so, where is it described?
Authors: The presented figures show GIMOD-air dried cells, however, all our samples were also critical point dried and showed the same pattern of surface features as demonstrated earlier [text ref. Gamliel et al, 1985]. The HCs were identified by comparative light microscopy, cytochemistry, biochemical markers, and TEM. The well-known Ficoll-Hypaque technique was used to separate the mononuclear band which consisted of lymphocytes, 50-95% hairy cells, and less than 5% monocytes.