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Hairy Cell Leukemia. Ultrastructural and Cytochemical Evaluation of Leukemic Colonies Grown in a Semi-solid Medium*

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Abstract—*We examined the fine structure and enzymatic activity of cells composing colonies grown from blood and/or spleen of eight patients with hairy cell leukemia. Mononuclear cells (MNC) were plated over an agar layer in a medium containing methylcellulose and leukocyte-conditioned medium. After* 7–10 days *incubation colonies were harvested for cytologic study. Colony cells possessed a euchromatic nucleus with an occasional nucleolus. Their cytoplasm contained a prominent Golgi region, numerous mitochondria and small vesicles, many short strands of rough endoplasmic reticulum (RER) and an infrequent phagosome. Well-developed ribosome-lamella complexes and what may have been their intermediate forms appeared in colony cells from three patients. Strong activity for tartrate-resistant acid phosphatase, localized in the RER, nuclear envelope and some Golgi vesicles, was evident in* 50–95% *of all colony cells. Our results indicate that a high proportion of MNC forming colonies in this culture system maintain the characteristic morphology and cytochemical activity of hairy cells.*

INTRODUCTION

HAIRY cell leukemia (HCL) is a malignant lympho-proliferative disorder of insidious onset, characterized by splenomegaly and the presence of hairy cells in the blood, spleen and bone marrow. Up to now, information concerning the *in vitro* growth and functional properties of isolated hairy cells has been limited by suboptimal culture conditions.

In a preceding paper we reported that mononuclear cells (MNC) from the blood or spleen of nine patients with HCL formed colonies in a supplemented medium containing methylcellulose [1]. Based on staining for tartrate-resistant acid phosphatase (TRAP), hairy cells were found in approximately 87% (range 50–95%) of the colonies grown in this system. Cloning efficiencies ranged from 0.03 to 0.32%, with a mean of 0.1%. No correlation was found between the composition of the colony population and cloning efficiency; source of cells or percentage of hairy cells in the plated sample also did not appear to affect the number and cellular composition of the colonies formed. In addition, three patients whose blood had been repeatedly sampled and showed similar percentages of hairy cells on each occasion produced variable numbers of colonies with different proportions of hairy cells.

In the present study we examine the fine structure and cytochemical properties of colony cells using new methods for the isolation and microscopic study of colonies formed in a semi-solid medium [2, 3]. The evidence presented confirms the malignant nature of these cells and suggests that some of the hairy cells in the original MNC population had clonogenic potential.

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MATERIALS AND METHODS

Mononuclear cells from blood and/or spleen homogenates of eight HCL patients were prepared and cultured in a cloning system as

detailed previously [2, 3]. Briefly, Ficoll-separated MNC were suspended in MEM-alpha medium (Gibco) containing 10% heat-inactivated fetal calf serum (FCS) and 1% penicillin/streptomycin at a concentration of 1.6×10^6 cells/ml. Immediately before plating, 0.5-ml aliquots of the MNC suspension were centrifuged and resuspended with 1.2 ml MEM-alpha medium, 0.8 ml phytohemagglutinin leukocyte-conditioned medium and 2.0 ml of a 1.6% solution of methylcellulose. One milliliter portions of this suspension (approximately 2×10^5 cells) were plated in three 35-mm Petri dishes containing an underlayer of MEM-alpha medium plus 10% heat-inactivated FCS and 0.5% agar. Cultures were incubated at 37°C in a humidified atmosphere plus 7.5% CO_2. After 7–10 days colonies (>80 μm diameter) were scored and then prepared for morphologic study.

We have described a simple method for the rapid isolation and concentration of leukemic colonies grown in media containing methylcellulose [2]. We employed this technique in the present study and found that the procedure was well tolerated by colonies; with gentle pipetting and centrifugations they remained intact and could be evaluated structurally and chemically. To summarize the method, colonies were harvested by reducing the viscosity of the upper colony layer with fresh medium alone. The colonies were carefully rinsed from plates and the contents of triplicate cultures combined in a single conical tube. Colonies were gently centrifuged (less than 10 *g*) and washed with medium to remove the methylcellulose. Following the final rinse, colonies were prepared for microscopic study.

From all cultures, smears and plastic-embedded specimens were prepared. Smears were prepared by cytocentrifugation at 750 rpm (50 *g*) for 8 min [2]; approximately 20 colonies were placed on each slide. Slides from each experiment were stained for cytologic evaluation according to the May-Grunewald-Giemsa method while others entered established protocols for the demonstration of peroxidase [4], non-specific esterase (NSE) [5] or TRAP [6].

After removal of part of the colony population for smears, fixative (2% glutaraldehyde + 0.8% paraformaldehyde in 0.2 M cacodylate buffer, pH 7.2) was added to the remaining suspension. Colonies were fixed for 30 min, washed with fresh buffer and post-fixed with buffered 2% osmium tetroxide for 1 hr. Following several buffer washes, they were pelleted in 2.5% agar (in buffer), dehydrated with graded ethanols and embedded in Epon 812 according to standard procedures. Semi-thin sections (1–2 μm) were stained with toluidine blue while ultra-thin sections were double-stained with uranyl acetate and lead citrate. Grids were examined with a Philips 300 transmission electron microscope at 60 V.

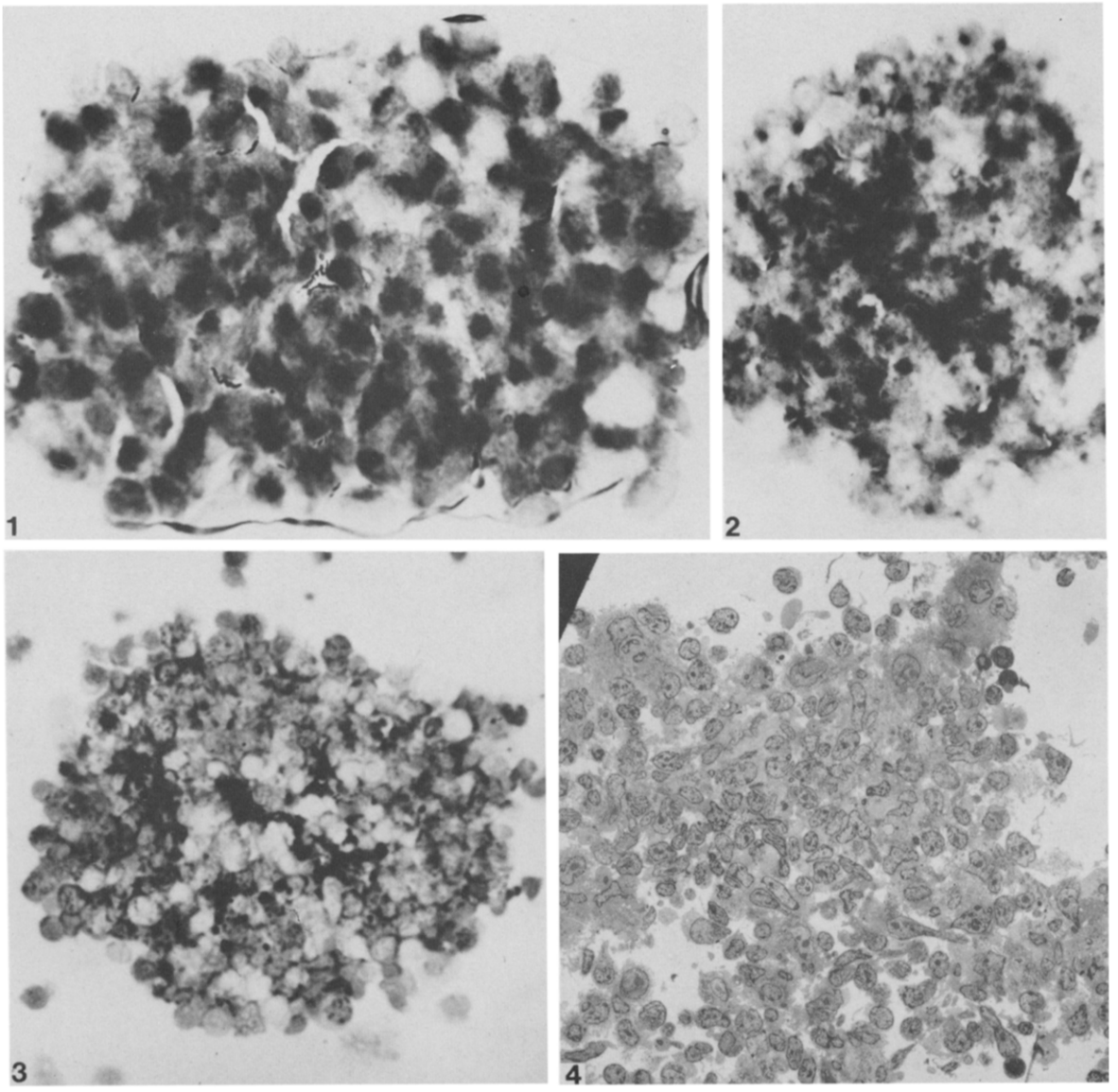
For the ultrastructural localization of TRAP, a portion of the colonies were segregated following primary fixation with aldehydes and treated with substrates containing naphthol-AS-BI-phosphate (Sigma) and lead tartrate. Reagents and procedures were identical to those outlined by Katayama and co-workers [7]. After the staining, colonies were post-fixed, dehydrated and embedded as described previously. Ultra-thin sections were examined without counterstains.

RESULTS

Hairy cells isolated from eight different patients formed 56–644 colonies (median 116) per plate after 7–10 days incubation. Colonies at this time were of various diameters (80–300 μm) and composed of densely aggregated cells. Due to the three-dimensional nature of colonies, the number and morphology of individual cells could only be ascertained with cytocentrifuge smears and plastic-embedded preparations of colonies (Figs 1–4). Smears were treated with a standard blood stain or exposed to specific substrates for the demonstration of TRAP, peroxidase or NSE. It was our experience that colonies with diameters greater than 200 μm smeared poorly so that resolution of their individual cells remained difficult (Fig. 3). This situation appeared to have a minimal effect on the cytochemical reactions, however, and the presence of enzymes in all colonies could be assessed (Figs 1 and 2).

Smears stained for TRAP showed activity for the enzyme in 50–95% of the cells in all colonies. The reaction product appeared as a homogeneous, bright red zone on the cytoplasm-side of the eccentric nucleus of colony cells (Fig. 1) [2]. Approximately the same percentage of cells showed activity for NSE, although the reaction product was less intense and covered a smaller area than had been seen with TRAP staining (Fig. 2). Reactions for peroxidase in colony cells were invariably negative.

The overall configuration of colonies was revealed in semi-thin sections of plastic embedded specimens. Generally, cells were loosely arranged at the periphery and became more densely packed as the core was approached (Fig. 4). Cell populations appeared homogeneous with respect to nuclear profile and cytoplasmic constituents. At the ultrastructural level, colony cells displayed many of the characteristic features of hairy cells, the most remarkable of which were their surface processes which projected freely or contacted an adjacent cell (Fig. 5). While these processes



Figs 1-3. *Cytocentrifuge smears of hairy cell colonies.*

Fig. 1. *Colonies stained for TRAP possess an homogenous red reaction product (black in this micrograph) in the majority of cells* (×1200).

Fig. 2. *Colonies treated for NSE show a similarly positioned but less intense reaction product (black in this micrograph)* (×550).

Fig. 3. *A large colony stained by the May-Grunewald-Giemsa method* (×450).

Fig. 4. *Semi-thin sections of colonies show cells to be loosely arranged at the periphery and packed tightly in the center* (×500).

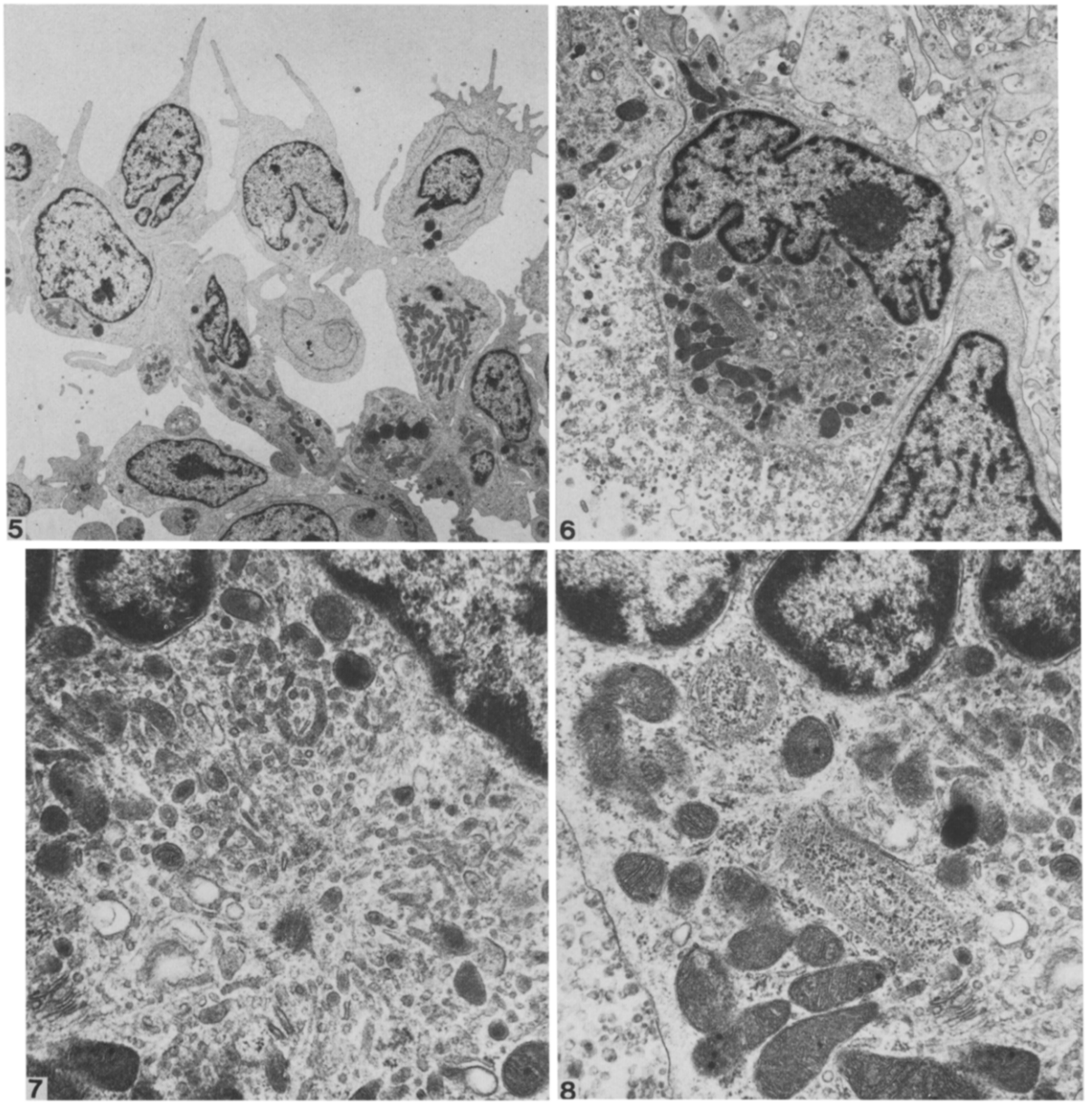


Fig. 5. Wide intercellular spaces at the colony margin permit visualization of the microvilli and larger processes typically found over the surfaces of hairy cells (×3000).

Figs 6–8. A series of micrographs of single cell near the center of a colony.

Fig. 6. Colony cells possess an indented, euchromatic nucleus with nucleolus (×7700).

Fig. 7. The area of cytoplasm adjacent the nucleus shows the greatest concentration of organelles. Large numbers of small vesicles appear in this micrograph (×30,000).

Fig. 8. Two R-L complexes are shown in transverse and oblique section. R-L complexes were observed in colonies of three patients (×30,000).

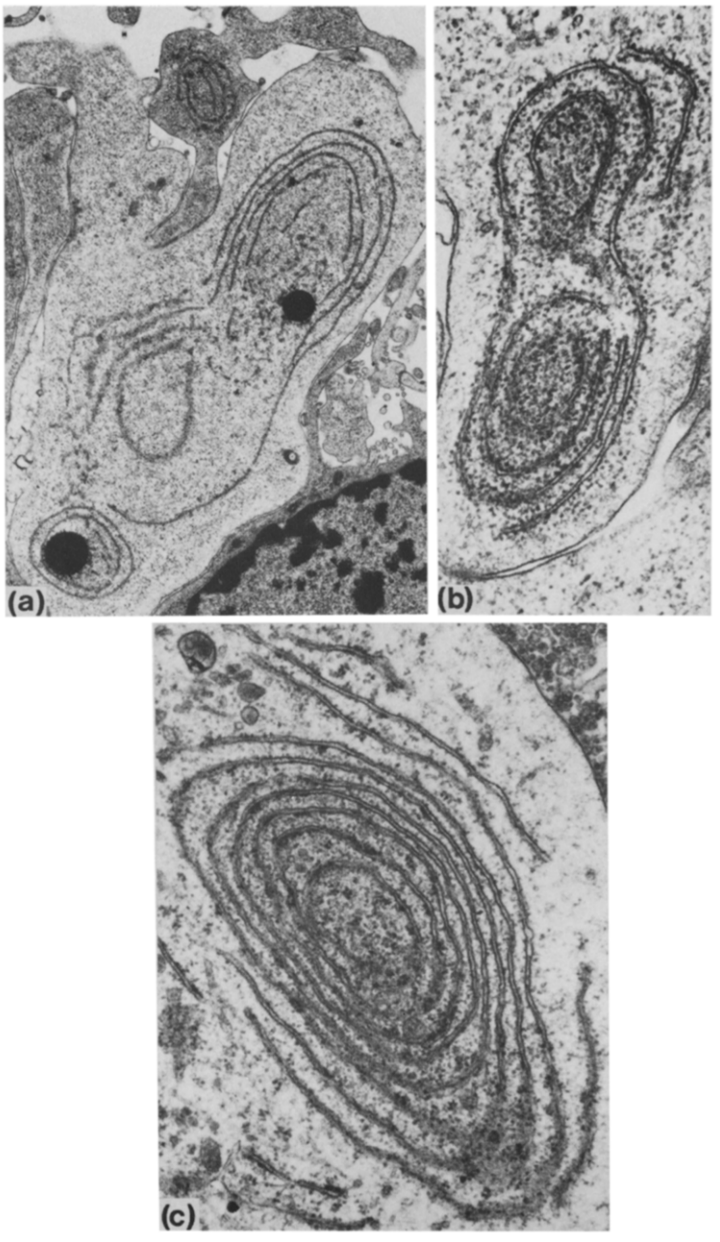
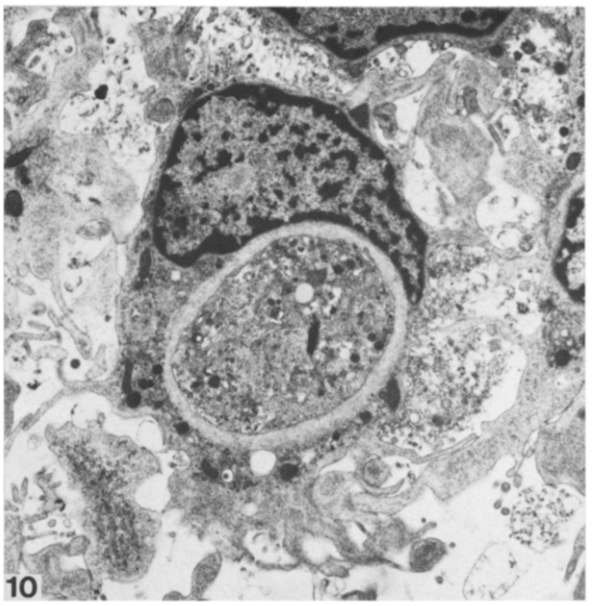


Fig. 9. A possible sequence of R-L complex formation is presented. (A) Short strands of RER with a loose, haphazard arrangement first appear. These strands may represent a long, single length of RER which passes in and out of the section plane (×10,000). *(B) The RER becomes coiled and more tightly aligned* (×31,000). *(C) The coil of RER is further condensed as the cytoplasm between membranes is reduced and the ER cisternae narrow* (×22,000).



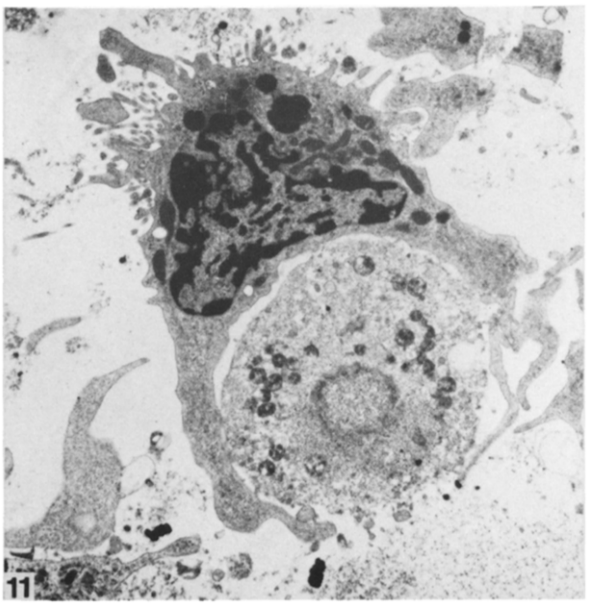


Fig. 10. *Phagosomes occasionally appear in colony cells. These membrane-bound vacuoles are surrounded by electron-lucent zones containing microfilaments* (×6500).

Fig. 11. *Hairy cells ingest debris by surrounding the target with their lamellapodia* (×6300).

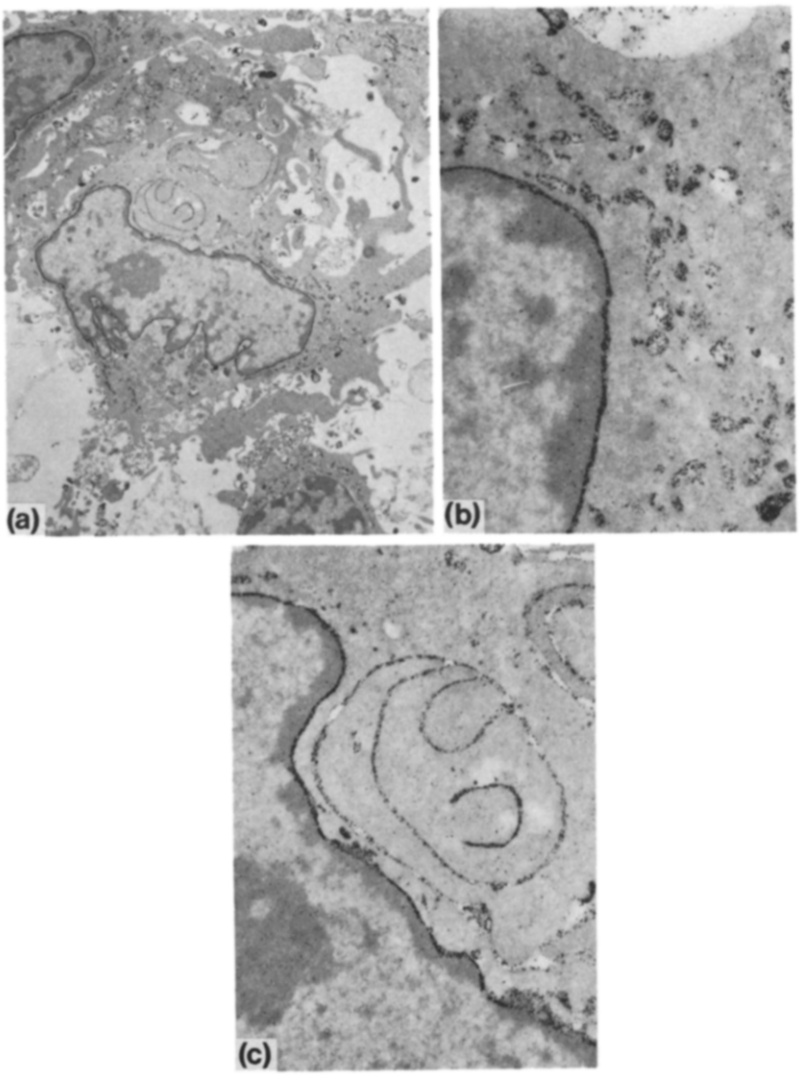


Fig. 12. A series of micrographs of a single colony cell stained en bloc for TRAP. (A) The majority of colony cells express the enzyme (black reaction product) (×4400). *(B) A higher magnification which shows TRAP localized in the nuclear envelope and small vesicles of the Golgi complex* (×26,000). *(C) Another field demonstrates the presence of TRAP in the cisternae of several strands of RER* (×16,000).

commonly had broad bases and tapered distally, intermittent swellings and branchings were often observed. Processes contained relatively few organelles; microfilaments and microtubules were present as well as an occasional mitochondrion or strand of rough endoplasmic reticulum (RER).

The nucleus of a colony cell was typically euchromatic with some peripheral chromatin condensation (Figs 4-6). A single, distinct nucleolus was present in the nucleus of most cells. Nuclear shape appeared dependent on the plane of section but usually showed at least one deep indentation and/or several smaller ones (Fig. 6).

Colony cells possessed a wealth of cytoplasmic structures; a fact demonstrated by the series of micrographs presented here showing a single hairy cell near the center of a colony (Figs 6-8). Because the nucleus is eccentrically positioned, almost all the organelles lie at the opposite pole (Fig. 6). Immediately adjacent to the nucleus on this side was the greatest concentration of organelles, including mitochondria, Golgi regions, centrioles, microtubules and numerous clear and electron-dense vesicles (Fig. 7). Scattered throughout the remainder of the cytoplasm were mitochondria, ribosomes, empty vacuoles, strands of RER and an occasional phagosome (Fig. 8). Microtubes were found in processes and throughout the cytoplasm of colony cells. Occasionally, pericentriolar satellites were observed in close association with the nucleus. These structures consisted of microtubules which radiated away from an amorphous, electron-dense core. Also near the nucleus were prominent Golgi complexes, composed of well-organized cisternae and small vesicles. Mitochondria were small but plentiful and possessed well-defined cristae and relatively dark matrices (Fig. 8).

One to several ribosome-lamella (R-L) complexes occurred in approximately 20% of the colony cells of three patients. In transverse section, R-L complexes possessed a hollow core and an outer sheath of multiple, concentrically arranged fibrils studded with a single row of ribosomes (Fig. 8). In oblique and longitudinal section, fibrils were seen aligned parallel with the long axis of the cylinder (Fig. 8). Rounded profiles of RER suggested that these organelles could play a role in the formation of R-L complexes. A possible sequence of intermediate steps in the development is presented in Fig. 9. The earliest stage consisted of a few aggregated strands of RER which passed in and out of the section plane (Fig. 9A). These strands became more concentric and an alignment of membranes became apparent as development progressed (Fig. 9B). The bundle of RER was consolidated by reduction of the cytoplasm between membranes as well as the diameter of the RER cisternae (Fig. 9C). The fact that the RER lamina was unbroken at this stage suggested that the organelle was a large cylinder with an elaborate, spiraling membrane. The ultimate step from RER to R-L complex appeared to be more subtle and/or faster than other stages and was not evident in any colony cells.

Evidence of phagocytosis by colony cells was occasionally apparent. Ingested material was sequestered in large, membrane-bound vacuoles which in turn were surrounded by an electron-lucent, filamentous zone (Fig. 10). In this area were layers of microfilaments which encircled the vacuole; an arrangement which suggested that the phagosome was walled off from the remainder of the cytoplasm. The uptake of debris by colony cells was accomplished by the extension and fusion of their lamellapodia around the target (Fig. 11). It was noteworthy that ingested cellular debris appeared unaltered and resembled matter in the extracellular space. Thus, while material was taken up by the typical phagocytic mechanism, once ingested it was not attacked by lysosomal enzymes.

Reaction for TRAP at the ultrastructural level revealed the enzymes' presence in the majority of colony cells: localized in the nuclear envelope, in small vesicles of the Golgi complex and in several strands of RER (Fig. 12). When phagocytic vacuoles were present, these inclusions invariably demonstrated no TRAP activity.

DISCUSSION

Blood and splenic mononuclear cells of HCL patients will form colonies when plated in a supplemented medium containing methylcellulose [1]. Using morphological and cytochemical methods, the present investigation establishes that the majority of colonies formed under these *in vitro* conditions are indeed of leukemic origin.

The unique morphology of hairy cells has been characterized by numerous light and electron microscopic studies [7-11]. In general, hairy cells most closely resemble normal lymphocytes and possess a large, indented nucleus with peripheral heterochromatin and a prominent nucleolus. Their cytoplasm is extensive and contains a large number and variety of organelles, giving the impression that the cells are metabolically active. In our investigations we made similar observations and, in addition, found that in colonies hairy cells had an eccentric nucleus and a high concentration of organelles adjacent to it. Especially prominent in this zone were many small vesicles which appeared to be empty or to

contain an electron-dense material. We also found that cells at the edges of colonies were loosely arranged so that their distinctive lamellapodia and microvilli projected freely into the intercellular space, making frequent contact with neighboring cells. Hairy cells in the center of colonies were more densely packed and their surface processes often interdigitated with those of other cells; an arrangement which mimics their *in situ* infiltration of the spleen and bone marrow.

Three cytochemical reactions were performed on isolated colonies to characterize their component cell populations. Strong activity for TRAP appeared in 50–95% of the cells in TRAP-positive colonies. This isozyme of acid phosphatase occurs in a variety of lymphocytic malignancies such as Sezary syndrome, Hodgkins disease, prolymphocytic leukemias, lymphosarcoma, Gaucher's disease, and T and B lymphoblastic leukemias [6, 12–16]. The enzyme appears almost exclusively in transformed lymphocytes and is considered to attain its greatest prominence and diagnostic significance in HCL [6]. The reaction product in colony cells was pronounced and appeared in the cytoplasm adjacent to the nucleus where organelles were plentiful. At the ultrastructural level, TRAP was localized in the nuclear envelope, in small vesicles of the Golgi region and in several strands of RER. Previous studies in other laboratories have shown similar activities and locations of TRAP in freshly isolated and cultured hairy cells [7, 17]. Non-specific esterase activity was evident in most colony cells. The reaction product was localized in the same region of the cytoplasm as the TRAP stain but was less intense and covered a smaller area. The presence of NSE in hairy cells has been documented and, while being of little diagnostic value, the reaction clearly differs from the strong, diffuse activity in normal and malignant cells of the monocyte series [11, 12, 18]. Peroxidase, another enzyme found in monocytic cells, was entirely lacking in colony cells. An absence of peroxidase activity in hairy cells has also been noted by others [19, 20]. The results of these cytochemical tests indicate that the HCL cultured from our patients was of lymphocytic origin and confirm that colonies were composed predominantly of hairy cells. The variable activity of TRAP may reflect stages in differentiation and/or a cell-cycle dependency of the enzyme [7].

We observed R-L complexes in colony cells of three HCL patients. These structures were identical to those which have been previously described in hairy cells [9–11, 21–24] and consisted of ribosome-studded fibrils which spiraled to form a cylinder. While R-L complexes cannot be considered diagnostically specific for HCL, having been observed in other forms of hemopoietic malignancies [21–23], they nevertheless show their highest frequency of occurrence in this type of leukemia [11]. In addition, since R-L complexes have never been reported in normal blood MNC [10], these structures at least serve as a useful marker of a malignant process. Our observation of rounded profiles of RER suggest that these organelles may play a role in R-L complex formation. Such a relationship has been put forward by others who have seen similar configurations of RER together with R-L complexes in malignant cells of patients with HCL [24] as well as other chronic leukemias [23] and adenoma [25]. Rosner and Golomb [24] observed that R-L complexes with ribosomes often had a continuous layer of rough endoplasmic reticulum immediately ajacent to the outer and innermost lamellar layers. They suggest that this organelle either actively contributes to the synthesis and maintenance of the R-L complex or the R-L complex as it enlarges causes adjacent endoplasmic reticulum to conform passively to its shape. Since it may be assumed that colonies are composed of cells in various states of differentiation, these arrangements of RER could represent initial sequences in the development of R-L complexes in hairy cells. The earliest stage appeared as a single strand of RER which passed in and out of the section plane and formed a loosely arranged coil. With continued development the coil became tighter due to a combined reduction in both the cytoplasm between the RER membranes and width of the RER cisternae. A step representing the dissolution of the RER with formation of the R-L complex was not apparent in any colony cells so that it remains unclear whether the membranes of the REF contribute directly to the fibrillar framework or serve only as templates for the proper alignment of microfilaments. Further research will be required if the relationship of RER to R-L complexes is to be clarified.

Phagosomes occurred infrequently in the cytoplasm of colony cells. These membrane-bound vacuoles were large and completely filled with cellular debris. An additional zone of low electron-density surrounded the entire organelle. This area was occupied by microfilaments which encircled the vacuole and appeared to wall it off from the remainder of the cytoplasm. Previous studies have reported that hairy cells show some capacity for phagocytosis *in vitro* and this in turn has lent support to the belief that HCL is a disorder of monocytic lineage [20, 26]. Hairy cells have been shown to ingest latex particles [20, 27], erythrocytes [9, 20] and microorganisms [21]. Jansen *et al.* [28], however, demonstrated with

lysostaphin and the extracellular tracer, lanthanum nitrate, that apparently ingested staphylococci were only cell-associated and not phagocytosed. Since we observed that the zone of microfilaments was apparent only in cells where debris was completely surrounded by cytoplasm and not just partially engulfed, we concluded that a few hairy cells were indeed phagocytic, although they did not behave entirely as would have been expected of true macrophages. At no time did lysosomes penetrate the microfilament zone and fuse with the vacuole; a finding substantiated by the apparent lack of any TRAP activity in phagosomes. Therefore, because secondary lysosomes failed to be formed, the contents of the vacuole escaped digestion and remained similar to debris in the extracellular space.

In summary, the present study confirms that the culture system outlined previously [1] promotes the clonal growth of hairy cells. The malignant nature of clones is verified by the fact that the majority of colony cells maintain the distinctive morphology and cytochemical traits of hairy cells. Also, research currently underway in our laboratory indicates that surface immunoglobulins expressed by clones are monoclonal with respect to their light chains [29]. The results also lend additional support to the opinion that most HCL have a lymphocytic origin since the enzyme patterns of colony cells differ from those of monocytes and unlike normal mononuclear phagocytes, hairy cells appear incapable of catabolising ingested material. This system, therefore, clearly enhances the *in vitro* clonal activity of hairy cells and should prove useful in monitoring the effects of modulators which influence their growth and function.

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REFERENCES

1. Hofmann V, Merchant R, Fehr J *et al. In vitro* cloning of hairy cell leukemia (HCL). *Proc ASCO* 1982, **1**, 136.
2. Merchant RE, Hofmann V. Human clonogenic cells *in vitro*. I. Improved preparatory techniques for the collection and concentration of leukemic colonies from methylcellulose cultures for morphologic, cytokinetic, and histochemical evaluation. *Stem Cells* 1982, **2**, 155–163.
3. Hofmann V, Merchant RE. Human clonogenic cells *in vitro*. II. Morphologic stability of cloned leukemic cells. *Stem Cells* 1982, **2**, 253–265.
4. Graham RC, Karnovsky MJ. The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. *J Histochem Cytochem* 1966, **14**, 291–302.
5. Yam LT, Li CY, Crosby WH. Cytochemical identification of monocytes and granulocytes. *Am J Clin Pathol* 1971, **55**, 283–290.
6. Katayama I, Yang JPS. Reassessment of a cytochemical test for differential diagnosis of leukemic reticuloendotheliosis. *Am J Clin Pathol* 1977, **68**, 268–272.
7. Katayama I, Li CY, Yam LT. Ultrastructural cytochemical demonstration of tartrate-resistant acid phosphatase isoenzyme activity in hairy cells of leukemic reticuloendotheliosis. *Am J Pathol* 1972, **69**, 471–478.
8. Ghadially FN, Skinnider LF. Ultrastructure of hairy cell leukemia. *Cancer* 1972, **29**, 444–452.
9. Katayama I, Li CY, Yam LT. Ultrastructural characteristics of the "hairy cells" of leukemic reticuloendotheliosis. *Am J Pathol* 1972, **67**, 361–370.
10. Katayama I, Schneider GB. Further ultrastructural characterization of hairy cells of leukemic reticuloendotheliosis. *Am J Pathol* 1977, **86**, 163–174.
11. Cawley JC, Burns GF, Hayhoe GFJ. Hairy-cell leukaemia. *Recent Results Cancer Res* 1980, **72**, 27–30.
12. Yam LT, Li CY, Finkel HE. Leukemic reticuloendotheliosis. The role of tartrate-resistant acid phosphatase in diagnosis and splenectomy treatment. *Arch Intern Med* 1972, **103**, 248–256.
13. Morse EE, Quinn J, Altman A. The use of leukocyte acid phosphatase in the diagnosis of malignant disease. *Ann Clin Lab Sci* 1980, **10**, 143–148.
14. Yam LT, Travassoli M, Jacobs P. Differential characterization of the "reticulum cell" in lymphoreticular neoplasms. *Am J Clin Pathol* 1975, **64**, 171–179.
15. Leder LD. Alkaline phosphatase and tartrate resistant acid phosphatase activity in cells of prolymphocytic leukemia. *Klin Wochenschr* 1978, **56**, 313–314.
16. Naeim, F, Capostagno VJ, Johnson CE *et al.* Sezary syndrome. Tartrate-resistant acid phosphatase in the neoplastic cells. *Am J Clin Pathol* 1979, **71**, 528–533.

17. Quan SG, Poolsawat SS, Golde DW. Ultrastructure and tartrate-resistant acid phosphatase localization in T-cell hairy cell leukemia cell line. *J Histochem Cytochem* 1980, **18**, 434-440.
18. Kass L. Nonspecific esterase activity in 'hairy cells'. *Acta Haematol* 1977, **58**, 103-107.
19. Yam LT, Castoldi GL, Garvey MB *et al.* Functional, cytogenetic and cytochemical study of the leukemic reticulum cells. *Blood* 1968, **32**, 90-101.
20. Palutke M, Weise RW, Tabaczka MT *et al.* Hairy cells and macrophages. A comparative study. *Lab Invest* 1978, **39**, 267-280.
21. Daniel MT, Flandrin G. Fine structure of abnormal cells in hairy cell leukemia, with special reference to their *in vitro* phagocytic capacity. *Lab Invest* 1974, **30**, 1-8.
22. Burke JS, Mackay B, Rappaport H. Hairy cell leukemia (leukemic reticuloendotheliosis) II. Ultrastructure of the spleen. *Cancer* 1976, **37**, 2267-2274.
23. Brunning RD, Parker J. Ribosome-lamella complexes in neoplastic haematopoietic cells. *Am J Pathol* 1974, **79**, 565-572.
24. Rosner MC, Golomb HM. Ribosome-lamella complex in hairy cell leukemia. Ultrastructure and distribution. *Lab Invest* 1980, **42**, 236-247.
25. Hoshino M. "Polysome-lamellae complex" in the adenoma cells of the human adrenal cortex *J Ultrastruct Res* 1969, **27**, 205-215.
26. Rozenszajn LA, Gutman A, Radnay J *et al.* A study of the nature of "hairy" cells, with emphasis on enzymatic markers. *Am J Clin Path* 1976, **66**, 432-441.
27. Burns GF, Cawley JC. A re-examination of the alleged monocytic features of hairy cell leukemia. *Scand J Haematol* 1979, **22**, 386-396.
28. Jansen J, Meijer CJLM, van der Valk P. Ottolauder GJ. Phagocytic potential of hairy cells. *Scand J Haematol* 1979, **23**, 69-79.
29. Arrenbrecht S, Moreillon M, Merchant R. Lymphocyte surface markers in cultured hairy cell leukemia. Proceedings of the 13th International Cancer Congress, 1982, 190.