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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 interspersed 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

are lymphocytes [Rubin et al, 1969; Ghadially & Skinnider, 1972; B-lymphocytes: Preud'Homme & Seligmann, 1972; Aisenberg et al, 1973; Burns & Hoak, 1973; Catovsky et al, 1974; Fu et al, 1974; Haak et al, 1974; Debusscher 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-naphtyl-acetate esterase activity, which is present in monocytes and histiocytes [Yam et al, 1971], has been demonstrated in HCs too [Schnitzer & Kass, 1974]. On the other hand, many reports have indicated that HCs belong to the lymphocytic series through cytochemical, morphological, and immunological studies. For example, HCs often contain tartrate-resistant acid phosphatase activity which is not present in other hematopoietic cells but has been demonstrated in atypical lymphocytes in cases of infectious mononucleosis [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 controversial early SEM results are discussed in the light of the current status on the unique SEM features of HCs.

SEM Studies Favoring the Monocytic Nature 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, phytohem-agglutinin 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 one 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 histiocyte/monocyte 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 by them as "non-B type HCL" showed a mixture of smoothly-undulated and ruffled surfaces. Based on these results, they suggested that hairy cells

might possess some membrane properties related to the monocytic series. It should be noted that some of their studied cells resembled the air-dried cells illustrated by Trubowitz et al [1971], therefore, the reliability of their actual drying technique and specimen coating procedure is questioned.

SEM Studies Favoring the Lymphocytic Nature of HCs

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 ruffled surfaces. Schnitzer & Hammack [1974] claimed that their results (i.e., the villous morphology of HCs) lent support to investigators advocating the B-lymphocyte nature for HCs.

Except for the fact that their cells were prepared for SEM by the CPD technique, the procedures used to fix, dehydrate, and coat the cells are not known. Although their photographs showed cells with what we interpret as optimally preserved surface details, they did not report a correlation between the percentage of villous cells seen under the SEM and the percentage of HCs in the spleen-cell suspensions assessed by light microscopy and histochemistry. As noted by Polliack et al [1974], it is possible that the cells illustrated were in fact 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 was 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 exclusively the characteristic HCs (over 90% abnormal cells), as judged by light microscopy and TEM. Using SEM, the spleen cells appeared 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.

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 and his colleagues [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].

HCs from different organ sites (i.e., bone marrow, spleen) were analyzed by Golomb & Simon [1977]. The surface ultrastructure appeared similar to that of HCs from the PB, regardless of whether the cells were examined directly in the tissue or from a prepared suspension of cells from the organ itself. It also did not seem to matter whether the cells in suspension were derived from either spleen or bone marrow. These results contrasted the marked differences between PB and spleen HCs suggested by Schnitzer & Hammack [1974], Roath & Newell [1975], and Catovsky et al [1975]. Golomb & Simon [1977] also pointed out that although there were some differences among patients in the shape of ruffles, most HCs had a surface ultrastructure which primarily consisted of exaggerated, broad-based, undulating ruffles with occasional areas of short microvilli. Although not stated, this description of surface features clearly indicated the lympho-monocytic hybrid nature of hairy cells.

Other supporting evidence for the 'hybrid' nature of HCs was provided by Katayama and Schneider [1977] who compared TEM and SEM studies on specimens from patients with LRE (HCL). In their studies, the blood samples obtained for observation were fixed immediately at room temperature by GA and Os to avoid in vitro changes of the leukocytes. After critical point drying the HCs displayed numerous cytoplasmic projections including short, stub-like microvilli, long microvilli, pseudopods, and ruffled membranes. These surface features distinguished the HCs from lymphocytes and monocytes, and were observed with both SEM and TEM. The stub-like microvilli were similar in size and shape to those seen in normal and leukemic lymphocytes which demonstrated fewer microvilli and no pseudopods. The ruffled membranes and long microvilli of the HCs seen by SEM corresponded in number, size, and shape with the pseudopods and microvilli seen by TEM.

Current Status

Recent functional, ultrastructural, immunological, cytochemical, and cytogenetic studies have lead 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, 1978; 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 reformative 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 alongside 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 leukocytes [Golomb & Reese, 1976; Dantchev & Belpomme, 1977; Polliack, 1977], contrasting the reports on cases with all-villous or all-ruffled types of HCs [Schnitzer & Hammack, 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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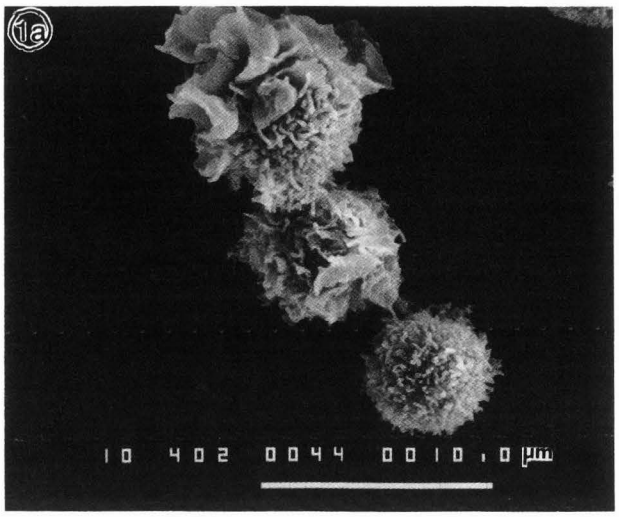

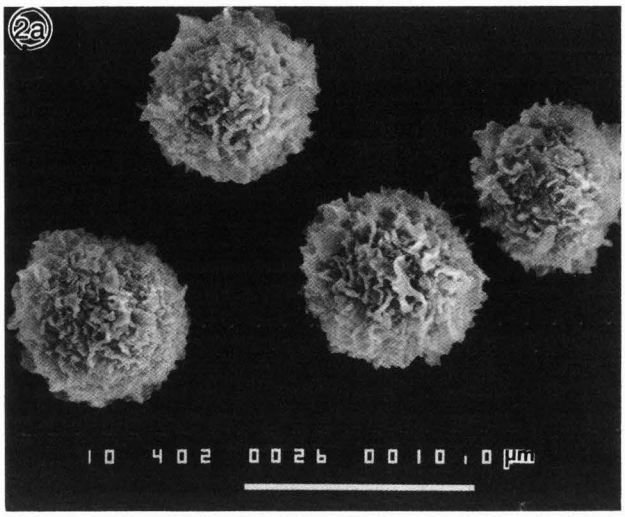

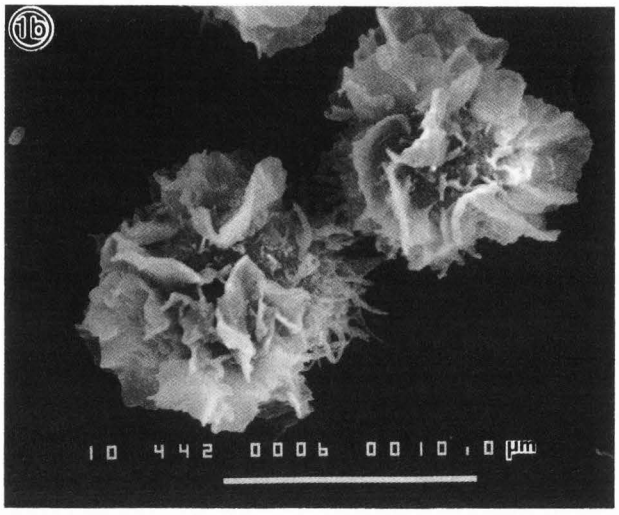

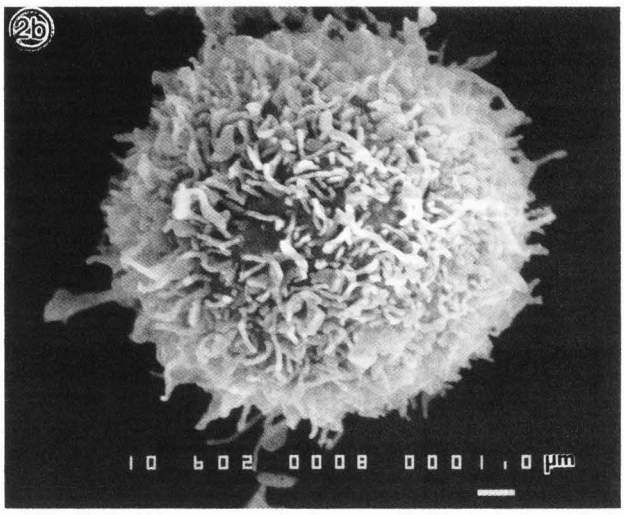

Figure 1: Type-A "hairy-cell leukemia" cells.
a. Two PB HCs (top) showing distinct clusters of microvilli and ruffled membranes.
b. Spleen HCs (from the same case shown in 1a) displaying numerous broad-based ruffles and clustered microvilli.

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

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Discussion with Reviewers

D. Catovsky: Have 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 [Polliack et al, 1984. In: "Human Leukemias" (ed. A. Polliack), pp 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 GTGO-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.