SEM observation of cultured, mitogen-stimulated leukocytes. Cells obtained from patients with acute myeloblastic and chronic lymphocytic leukemias[1]

E.D. Lalague, A.A. Abugaber, S. Haviernick, M.-R. Corvellec, L. Pelletier, C. Derghazarian and G.H. Cousineau

Laboratoire de Biologie Moléculaire, Département de Biologie, Université de Montréal, C.P. 6128, Montréal, Québec, H3C 3J7, and the Service d'Hématologie, Hôpital Ste Jeanne d'Arc, 3570 St. Urbain, Montréal, Québec H2X 2N8 (Canada), 16 September 1980

Summary. SEM observation of acute myeloblastic leukemia cells, incubated for 20 h with the mitogens pokeweed and phytohaemagglutinin, showed these to have elongated structures that were either smooth or partially covered by thumblike figures. By contrast, the chronic lymphocytic leukemia cells possessed more compact shapes and some were covered with blebs of varying sizes.

We have recently reported a modified technique for the isolation of human mononuclear leukocytes from peripheral blood, and the preparation of these cells for observation with a scanning electron microscope (SEM)[3]. The purpose of the present communication is to report on the general surface architecture, as revealed by SEM, of mononuclear leukocytes isolated from whole, peripheral human blood and incubated for 20 h in the presence of 2 mitogens, pokeweed (PWM) and phytohaemagglutinin (PHA). The cells studied were obtained from normal individuals, from one case of acute myeloblastic leukemia (AML) and one case of chronic lymphocytic leukemia (CLL).

Materials and methods. Total heparinized blood was obtained and treated as previously described (Ficoll-Hypaque gradient)[4]. The pelleted cells were suspended in Hanks' buffer containing 0.04 M sucrose, recentrifuged, and the concentration adjusted to 3×10^6 cells ml^{-1}. From this suspension one portion of material was immediately used for SEM (JSM-35) observation[3] and the other was utilized for the culture of leukocytes, as described below. The AML patient had a total white blood cell count of 12,600 cells ml^{-1} with the following differential: blasts, 54%; segmented cells (neutrophils), 41%; monocytes, 1%, and lymphocytes, 3%. The CLL patient had a total white blood cells count of 25,500 cells ml^{-1} with the following differential: lymphocytes, 81%; segmented cells (neutrophils), 17%; eosinophils, 1%, and basophils, 1%.

Culture of leukocytes: the cells were grown either with mitogens (stimulated cells) or without mitogens (non-stimulated cells). The growth medium for the stimulated cells contained the following: Hanks' buffer (29 ml); autologous serum (5 ml); pokeweed (0.5 ml); phytohaemagglutinin (0.5 ml); penicillin, 1000 IU ml^{-1} and streptomycin, 1000 µg ml^{-1} (0.5 ml). 5 ml of cells (suspended in Hanks' buffer) were very gently added to 45 ml of culture medium in a 250-ml DeLong culture flask, into which polylysine-coated coverslips had been introduced. The leukocytes were then incubated for 20 h without agitation at 310 °K. The coverslips, carrying the settled leukocytes, were then removed and the cells washed for 3 min in Hanks' buffer (pH 7.4 and containing 0.9% NaCl and 0.04 M sucrose) at 296 °K. The cells were finally fixed for 30 min at 296 °K with 4% glutaraldehyde, made up in Hanks' buffer + 0.04 M sucrose, and prepared for SEM observation.

Results and discussion. Fresh normal leukocytes: the observation of normal, peripheral mononuclear leukocytes[5] revealed the presence of typical microvilli-covered lymphocytes and some large, irregular monocytes with their striking ruffles.

Cultured normal leukocytes: after these cells had been cultured for 20 h in autologous plasma, the microvilli were lost, and the lymphocytes remained free, taking a regular, rounded configuration.

Cultured normal leukocytes with mitogens: when the leukocytes had been incubated in the presence of the mitogens PWM and PHA the following general configurations could be observed: cells with a large anterior extension of velum-like, cytoplasmic material; cells covered by irregular nodosities and with uropodia at one end (surrounded by filipodia); cells with uropodia at one end and tufted cellular extremities at the other; mirror-like configurations and inter-cellular cytoplasmic bridges. All these configurations have been reported by a number of laboratories[3,5-7], and

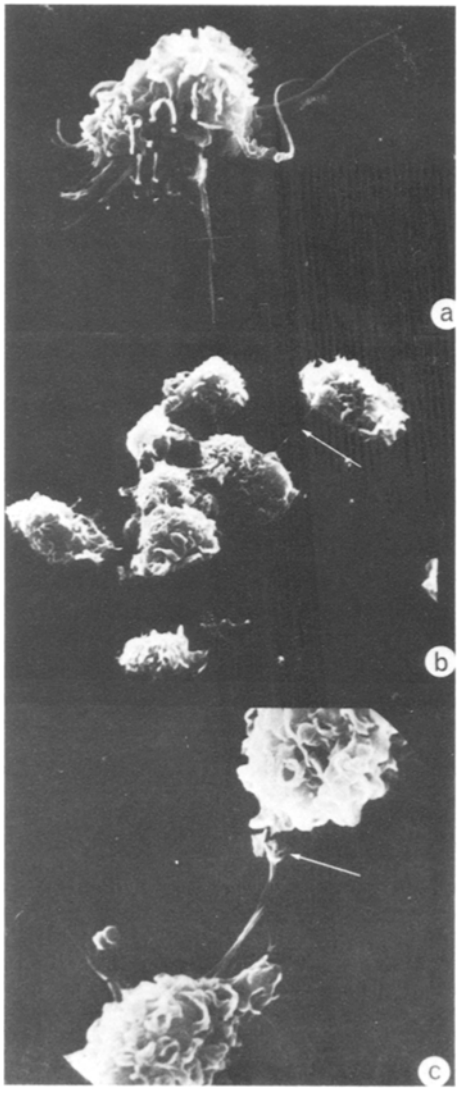

Figure 1. Normal leukocytes in culture for 20 h in the presence of PWM and PHA. Note *a* the spider-shaped cell (×8400), *b* the cytoplasmic bridge (×4200) and *c* the sucker-like extension (×9600).

only a few examples are given with figure 1. It is important to indicate that the use of 2 different lectins, namely PHA stimulating T-cells and PMW stimulating both B- and T-cells, was not performed in order to distinguish between cells types, but rather to verify how these mitogens could be used simultaneously and what general leukocyte morphology could be observed.

Fresh leukemic leukocytes: these cells were treated at the same time and in the same manner as the normal cells. Each sample of fresh peripheral mononuclear leukocytes that was examined (cells from either AML or CLL preparations) revealed the presence of what appeared to be aggregates of precipitate (fig. 2a). Although never found associated with normal cells, this material was regularly seen associated with the leukemic cells. The cells themselves in the AML (fig. 2a) and CLL (fig. 2b) samples were found to belong mainly to the smooth category[8] (over 80% of the leukemic cells had a smooth surface, whereas over 80% of the cells in normal preparations showed finger-like projections). The other most common configurations observed were lymphocytes with markedly to moderately villous surfaces (fig. 2, c and d), and in most cases the villi appeared stub-like.

Cultured leukemic cells: after the leukemic cells had been cultured for 20 h, the most frequently observed configurations were again the spherical, smooth-surfaced cells in both the AML and CLL samples, but other configurations were also seen, such as cells having a hand-mirror configuration and elongate cells with areas of clustered ruffles, among others (fig. 3), and again all these configurations were observed in both AML and CLL samples.

Cultured leukemic cells with mitogens: when the leukemic cells had been cultured for 20 h with PWM and PHA some differences in general surface architecture between AML and CLL samples seemed to appear. Indeed, the AML cells developed mostly into elongated structures that were either

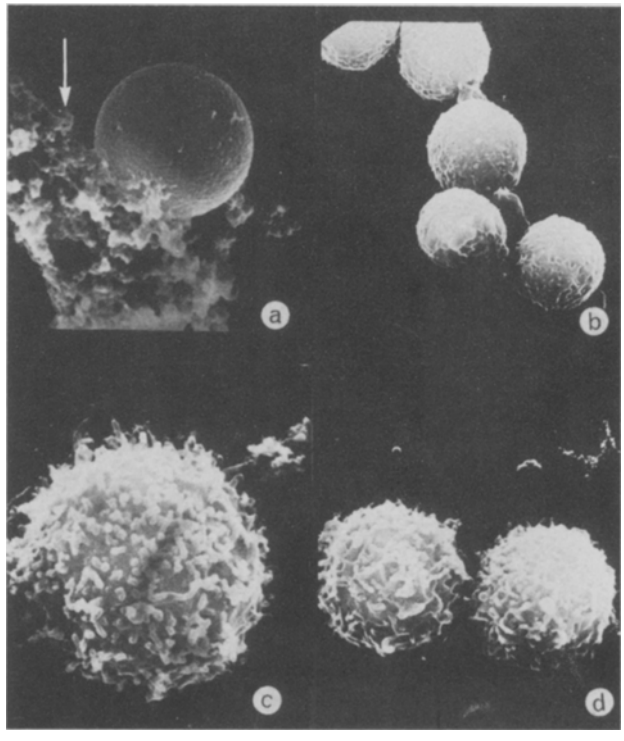

Figure 2. Mononuclear leukocyte preparations from fresh, peripheral blood. *a*, smooth cell from AML samples. Precipitate material at arrow (× 5000); *b*, smooth cells from CLL preparations (× 2400). Markedly to moderately villous lymphocytes observed both in AML (*c*, × 7500) and CLL (*d*, × 5000) samples.

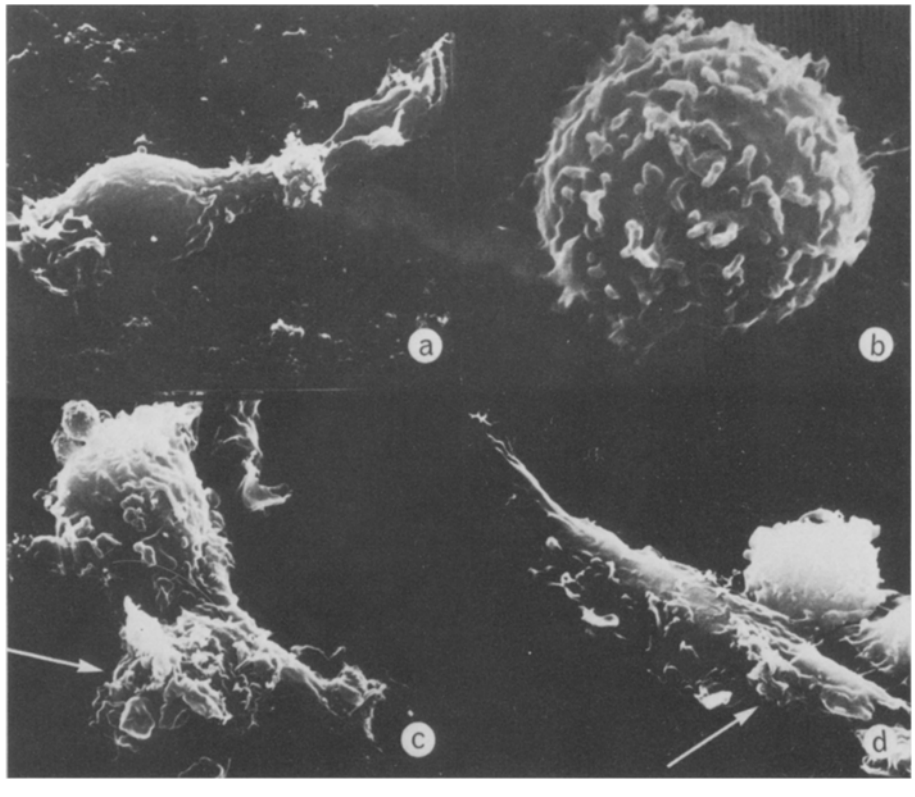

Figure 3. AML cells after 20 h of incubation in autologous plasma without the mitogens PWM and PHA. *a* Hand-mirror configuration with tufted extremity (× 3700); *b* non-transformed lymphocyte (× 9200) and *c* and *d* elongated cells with areas of clustered ruffles (arrows) (c, × 4000 and d, × 2950).

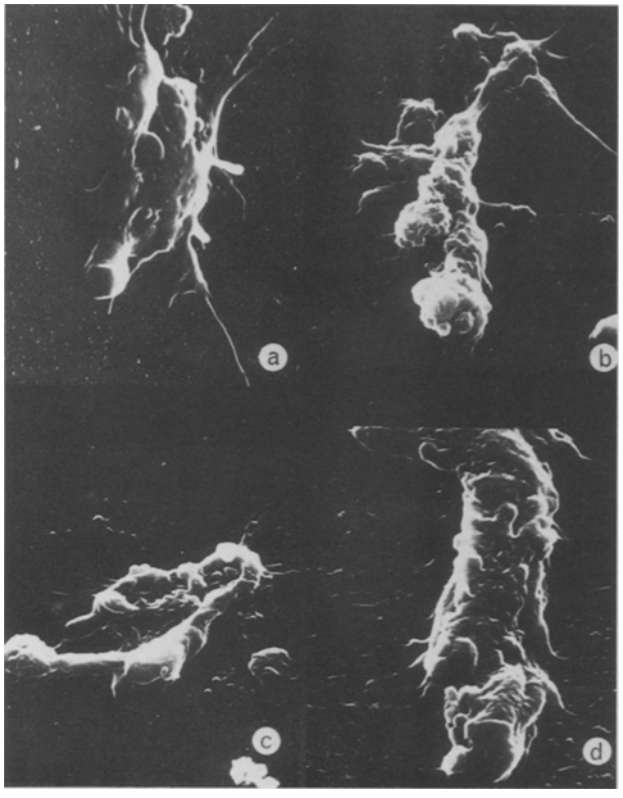

Figure 4. AML cells, after 20 h of incubation in autologous plasma with the mitogens PWM and PHA, showing elongated configurations with thick, cytoplasmic extensions, often covered with thumb-like structures. Some cells also possessed very dense areas (*a*, ×4300; *b*, ×5000; *c*, ×3600 and *d*, ×6500).

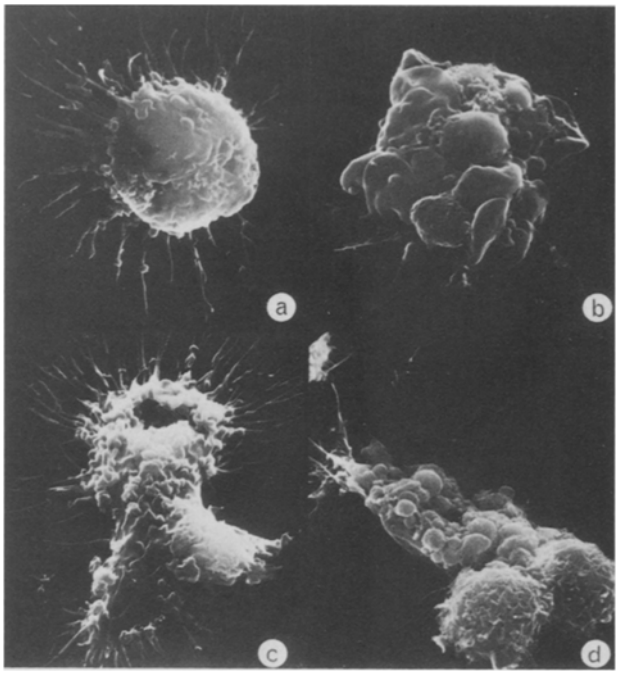

Figure 5. CLL cells, after 20 h of incubation in autologous plasma with the mitogens PWM and PHA, generally showing compact configurations. Some cells were covered with blebs of varying sizes (*a*, ×3300; *b*, ×5000; *c*, ×2700 and *d*, ×3000).

smooth or partially covered by thumb-like figures or projections, other cells were observed to contain thickened areas (fig. 4). On the other hand, CLL cells showed a generally more compact shape, and some were covered with blebs of varying sizes (fig. 5).

It has been assumed that the configurations observed, after incubation of these cells in the presence of PWM and PHA, result from stimulated lymphocytes, but it must be noted that the Ficoll gradient method also allows the extraction of blast cells[9] as well as monocytes, and the possibility cannot be excluded that perhaps non-leukemic monocytes could be stimulated by lectin-stimulated lymphocytes present in the cell suspension. Nevertheless, the configurations observed were typical of the samples examined, and the only cells observed in fresh samples were smooth surface, or villi-covered, spherical cells and monocytes.

We realize that the data presented herein, originating from only 2 patients (although similar results, not reported here, were obtained from a 3rd leukemic patient) are quite limited, but we nevertheless feel confident of the following proposition; if we allow that the general biochemical differences that may exist between normal and leukemic cells are reflected in their morphological profiles then these differences, if sufficiently enhanced with the use of PWM and PHA, could possibly be noted by the use of SEM: this could prove a potentially valuable addition to the cell culture studies of Senn et al.[10]. The methodology presented could perhaps become a useful tool in the early diagnosis of leukemia.

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4 The products used for the culture of leukocytes were as follows: Hanks: Hanks' balanced salt solution, GIBCO cat. No. 310-4020 and lot No. 91-1011; Pokeweed: mitogen (PWM) (Barker and Farnes), used as recommended by GIBCO: 1.0 ml of rehydrated mitogen to 100 ml of culture medium, cat. No. 670-5360 and lot No. R-293012; Phytohaemagglutinin: (PHA) (M Form), used as recommended by GIBCO: 1.0 ml of rehydrated mitogen to 100 ml of culture medium, cat. No. 670-0576 and lot No. A782223; Penicillin-G: 1667 units mg^{-1}. SIGMA Chemical Company, cat. No. PEN-NA and lot No. 44C-1150; Streptomycin sulfate: SIGMA Chemical Company, cat. No. S-6501 and lot No. 92C-1210.
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