IDENTIFICATION OF HUMAN B AND T LYMPHOCYTES BY SCANNING ELECTRON MICROSCOPY*

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It is now generally accepted that two distinct classes of lymphocytes are present in different species including man—thymus-derived (T) cells and bone marrow or bursal-derived (B) cells (1, 2). Although similar in appearance these two cell types have been demonstrated to have widely different functions. They can be distinguished electrophoretically by their different mobility (3, 4), serologically by the presence or absence of specific surface antigen markers (5–7), and by their different in vitro reactivity to various lectins (8, 9). B cells are readily recognized by surface immunoglobulin staining (10), the presence of Fc receptors detected by uptake of aggregated γ-globulin (11), and by a complement receptor (12). Recently it has been demonstrated that T cells in the human have the specific property of forming rosettes with sheep red blood cells. This simple procedure is finding wide application for the recognition of T cells (13, 14).

Electron microscope observations, utilizing ferritin (15), virus, or peroxidase markers (16, 17), and autoradiography (18) have been used to identify certain of the surface components of B and T cells. However, the distinction between B and T-derived cells has depended so far primarily on these immunologic methods, since by routine light and transmission electron microscopy, these two types of lymphocytes have not been clearly distinguished (14, 19–21).

Before the present investigations, scanning electron microscopy (SEM)[1] has not been specifically applied to the study of B and T lymphocytes. Generally, blood cells have been examined by SEM after air drying, a technique known

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[1] *Abbreviations used in this paper*: CLL, chronic lymphocytic leukemia; HBSS, Hanks' balanced salt solution; Ig, immunoglobulin; PBL, peripheral blood lymphocytes; SEM, scanning electron microscopy; SRBC-R, sheep red blood cell rosettes.

to cause distortion of surface detail (22, 23). The aim of the current study was to examine the surface architecture of a variety of human lymphocytes, of known B or T derivation, prepared for SEM by the critical point drying method, in an attempt to define specific differences between the two types of lymphocytes.

Materials and Methods

Samples.—The following samples were examined by scanning electron microscopy: peripheral blood lymphocytes (PBL) from seven normal individuals, buffy coats from six untreated patients with chronic lymphocytic leukemia (CLL), thymocytes obtained from two normal individuals, and four cultured lymphoblastoid cell lines—SK-L7, SK-LN (24), 8866 (25), and MOLT-4 (26).

Buffy Coats.—These were obtained by centrifugation of anticoagulated venous blood at 1,000 rpm for 8 min at room temperature or as direct buffy coats after leukophoresis on an IBM cell separator. A minute portion of the buffy coat was removed with a Pasteur pipette and resuspended in isotonic saline (pH 7.4, room temperature). The suspension was then centrifuged at 1,000 rpm for 8 min, the supernatant removed, and the pellet resuspended in normal isotonic saline.

Separation of PBL.—The Ficoll-Hypaque density gradient method (27) was used to separate lymphocytes and monocytes from other leukocytes in most normal samples. Two samples were separated by additional filtration through nylon fibers (28). The cells were washed two to three times in isotonic saline or Hanks' balanced salt solution (HBSS) and resuspended in the same solution.

Thymocytes.—Fresh human thymus was obtained from two young patients with Fallot's tetralogy, while undergoing cardiac surgery. The tissue was minced, and passed through a wire mesh and then cells were processed as for PBL.

Cultured Cells.—SK-LN and SK-L7 lymphoblastoid cell lines (24) were grown in suspension culture (logarithmic and stationary phase of growth) and maintained in McCoy's medium supplemented with 30% fetal calf serum. Suspension cultures of the 8866 cell line (25) were kindly provided by Dr. S. Gordon, The Rockefeller University, New York, and maintained in Dulbecco's medium with 10% fetal calf serum (logarithmic and stationary phase of growth), and suspension cultures of the MOLT-4 cell line (26) were kindly provided by Dr. J. Minowada, Roswell Park Memorial Institute, Buffalo, N. Y., and maintained in RPMI-1640 medium with 10% fetal calf serum (logarithmic and stationary phase). Cell suspensions were centrifuged twice at 1,000 rpm for 5 min and the cell pellet was resuspended in fresh culture media without fetal calf serum.

Preparation of Specimens for SEM.—

Harvesting, fixation, and dehydration of cells: Cells were collected by aspiration-filtration on "Flotronic" silver membranes (Flotronic, Inc., Spring House, Pa.) of 0.45 or 0.8 μm porosity, as described in an earlier study (29). The silver membranes layered with cells were fixed immediately for at least one-half h with 1% glutaraldehyde (pH 7.3, 320 mosmol), rinsed twice with buffer (pH 7.3, 320 mosmol), postfixed in 1% osmium tetroxide (pH 7.3, 320 mosmol) for an hour, subsequently rinsed twice in buffer, and dehydrated in a graded series of alcohol for 5 min each. Premature drying of the cells was always carefully avoided by never aspirating completely any of the fixation or dehydration fluids. The silver membrane was then prepared for critical point drying in carbon dioxide, as described by Anderson (30).

Critical point drying: Dehydration of the sample was continued further through a graded series of amyl acetate/absolute alcohol and then absolute amyl acetate for 5 min each. The soaking specimen was quickly transferred to a previously cooled high-pressure chamber

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(Denton critical point apparatus[2]). Carbon dioxide was vented through the chamber for 10–15 min, the pressure within the chamber reaching 800–900 lb/in^2 at this stage. When there was no detectable odor of amyl acetate at the level of the exhaust valve, the two valves were tightly closed and a water bath was used to raise the temperature of the chamber to 50°C, the pressure reaching approximately 1,500 lb/in^2. The chamber was vented slowly and the dried specimens were removed.

Coating: Portions of the membrane were attached to stubs using double-sided sticky tape and coated with a thin layer of carbon and gold on a rotatory stage (Denton vacuum apparatus, DV-502 [Denton Vacuum, Inc.]) at an angle of 15°. Under standardized conditions the gold deposition averaged 250 Å as calculated by nomograms and the carbon film 120 Å as checked by direct densitometry to standards measured by a crystal quartz thin film monitor. The specimens were then stored under vacuum until examination.

Examination of Specimens.—A Cambridge S4 scanning electron microscope[3] was used, at an accelerating voltage of 20 kV, and with 100 or 200 μm diameter illuminating apertures. Micrographs were recorded on Polaroid type 55 P/N films at direct magnifications from × 1,000 to 14,000. Resolution of the SEM, tested on electrostatic copying paper and magnetic tape, was in the order of 160 Å. The microscope was calibrated using a 400 mesh copper grid mounted on a stub as a calibration specimen. Hundreds of cells were scanned on the SEM screen in order to obtain an evaluation of cell size and surface morphology of the entire population before recording micrographs. Approximately 7–8 × 10^6 are required to give a monolayer-like distribution of cells on a 25 mm diameter silver membrane, and in this study samples with approximately from 2 × 10^6 to 1.2 × 10^7 cells per membrane were examined. A few random low-magnification micrographs were recorded from each specimen at × 500–2,000 direct magnification for initial counting purposes. Between 60 and 120 cells appear on each micrograph at these magnifications depending of course on the initial concentration of the sample. For more detailed observation of surface architecture, micrographs were taken at direct magnifications between × 5,000 and 14,000. Measurements were not done on stereopairs, but cells were finely traced out and their diameters and measurements of surface digitations carefully recorded in order to indicate a size range.

Preparation of Sheep Red Blood Cell Rosettes (SRBC-R).—Rosettes were prepared according to the method described in an earlier study (14). 0.1 ml of 0.5% SRBC in HBSS, 0.1 ml of lymphocyte suspension (5 × 10^6 cells/ml), and 0.02 ml of heat-inactivated pooled human AB serum (Grand Island Biological Co., Grand Island, N. Y.), which had been previously absorbed with an equal volume of packed SRBC or of HBSS, were mixed thoroughly and incubated at 37°C for 5 min. The mixture was then centrifuged at 50 *g* for 5 min at room temperature and incubated at 4°C for 60 min. The cell pellet was resuspended by very gently rocking the tubes. A minimum of 200 lymphocytes was counted in a hemacytometer and lymphocytes were counted as rosette positive if any SRBC were adherent to them.

Fluorescence Microscopy.—Immunofluorescence staining of lymphocyte surfaces for immunoglobulin and for aggregated human immunoglobulin was performed as described previously (11, 31). Polyvalent antihuman immunoglobulin antiserum and aggregated human immunoglobulin, both conjugated to tetramethylrhodamine isothiocyanate, were used for staining of surface immunoglobulin and aggregate binding, respectively.

Separation of Rosettes from Nonrosetting Cells.—0.4 ml of SRBC-R-containing suspension was layered on 1.5 ml of the Ficoll-Hypaque mixture used for lymphocyte isolation and centrifuged at room temperature for 20 min at 400 *g*, at the interface between the cell suspension and the Ficoll-Hypaque mixture. The lymphocytes at the interface were carefully pipetted

[2] Denton Vacuum, Inc., Cherry Hill, N. J.

[3] Kent Cambridge Scientific Inc., Morton Grove, Ill.

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off and the percentage of rosettes and immunoglobulin staining cells in both this layer and the bottom layer was counted.

RESULTS

Normal Peripheral Blood Lymphocytes (PBL).—After examination of over a thousand cells by SEM and actual counting of close to a thousand cells from micrographs of different samples, it appeared that one could generally recognize two cell types and subdivide normal PBL into two broad groups on the basis of their surface morphology (Fig. 1). About one-fifth of the cells had a complex villous surface architecture, whereas the surface of the majority of PBL was generally smooth with or without a small-to-moderate number of surface digitations.

"Villous" lymphocytes (Fig. 2): These cells, ranging from 5.1 to 6.4 μm (average 5.8 μm) in diameter, constituted from 7.6 to 34% (average 20.3%) of PBL examined. They were identified by their complex surface architecture, with multiple microvilli covering almost the entire surface. There were from 90 to 257 (average 152) microvilli per exposed cell surface (half the total surface), varying in length up to 900 nm (occasionally up to 1.7 μm) and in breadth from 70 to 230 nm (with some up to 350 nm).

"Relatively smooth" lymphocytes (Figs. 3–6): These cells were also spherical but smaller in diameter, ranging from 4.0 to 5.0 μm (average 4.5 μm) and constituted 65–92% (average 79.7%) of PBL examined. Approximately 40% of these cells had a generally smooth surface without obvious surface projections (Fig. 3). These cells were often attached to the substrate by fine extensions of cytoplasm. The remaining cells had basically the same surface architecture but from 1 to 25 (average 8) digitations per exposed surface were present (Figs. 4 and 5). These were either "stub-like" (35–55 nm broad, occasionally up to 210 nm) or shorter microvilli (100–350 nm broad, and generally up to 350 nm and rarely 720 nm long) with the surface between the microvilli readily visualized. These differential counts were made at low magnifications. However, at higher magnifications, a small group of cells (approximately 6–14%, Fig. 6) showed a somewhat larger number of surface digitations (25–72, average 53 per exposed cell surface), most of which were stub-like in appearance (50–110 nm broad) and about a third microvilli (210–720 nm long). Although difficult to classify with certainty, these cells were thought to be related to the relatively smooth type because of their smaller size, general surface architecture, and relatively small average number of surface digitations. However, it is difficult to exclude the possibility that they are in fact, a separate group of smaller villous cells having less surface digitations than the average villous cell.

Immunologic data: Using the SRBC rosette formation as a T cell marker and positive staining with either anti-Ig or aggregated γ-globulin, as B cell markers, between 69 and 82% of PBL from these normal individuals were identified as T cells and from 20 to 30% as B cells. A small population of cells, approximately

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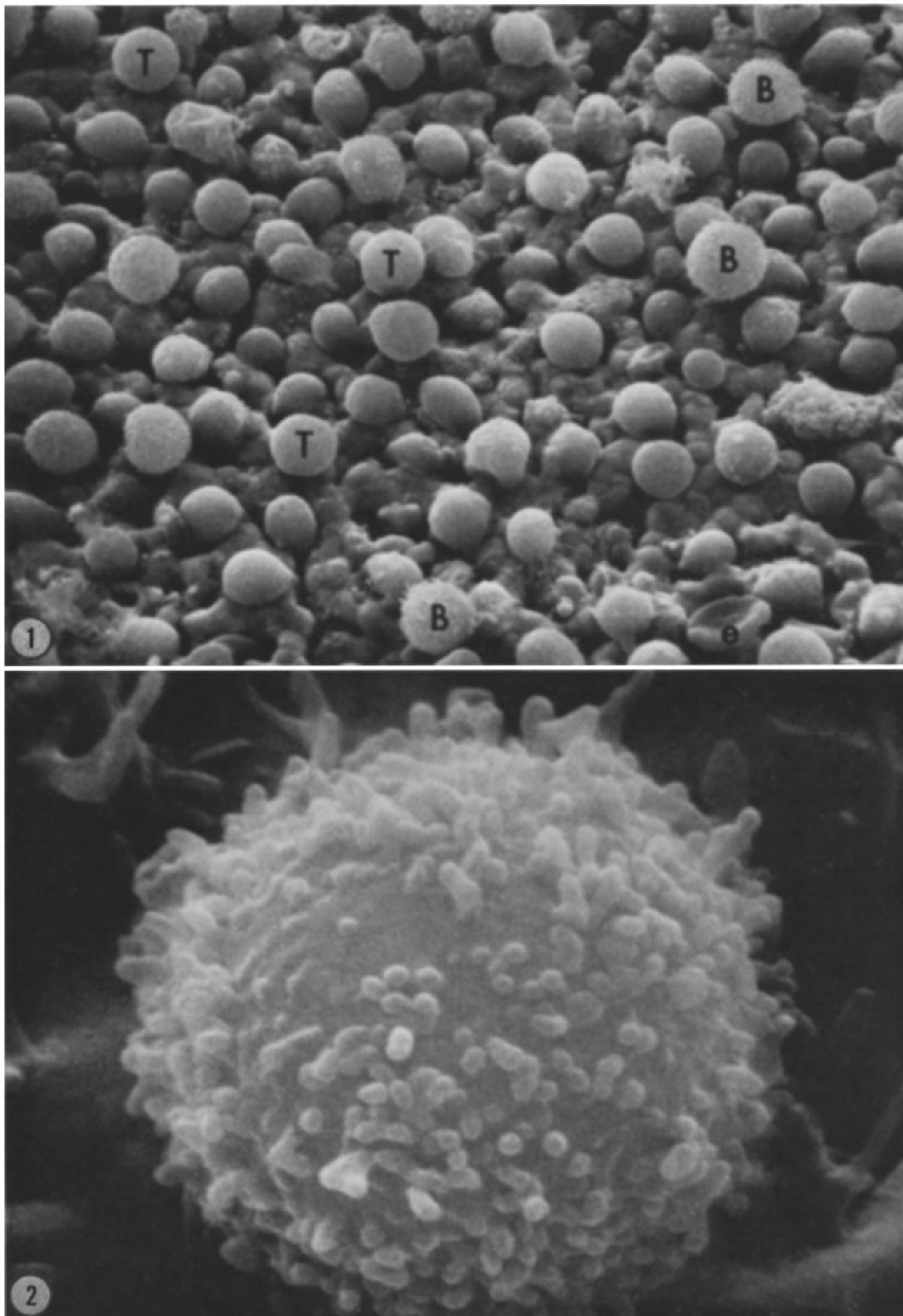

FIG. 1. Low-magnification micrograph of PBL, taken for counting purposes, showing the two types of PBL. The majority of cells have a relatively smooth surface (*T*) and a smaller number of villous cells are present (*B*). A single erythrocyte (*e*) is seen. × 2,250.

FIG. 2. Typical normal B lymphocyte, with a complex villous surface pattern. × 18,000.

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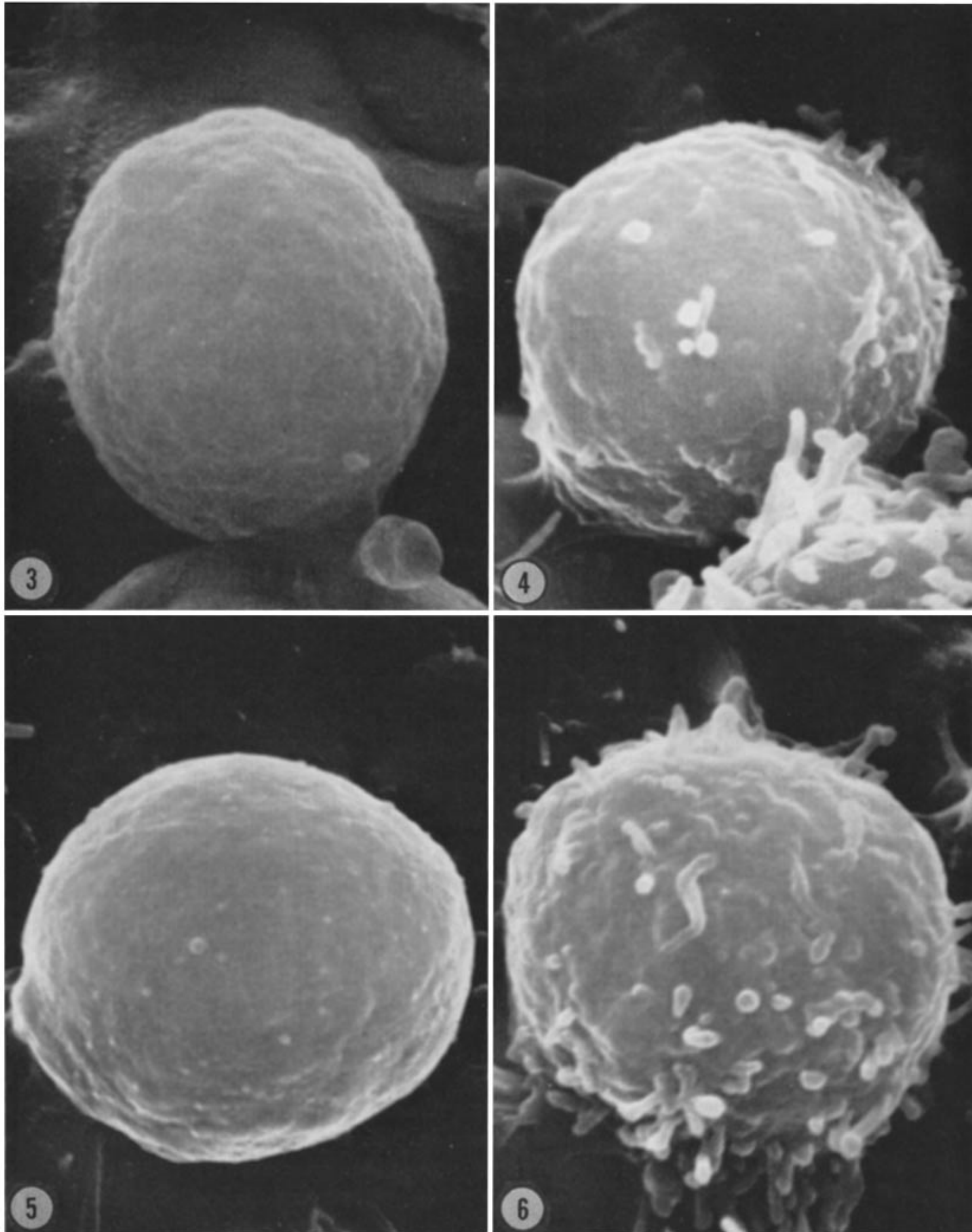

FIGS. 3–6. Spectrum of normal T lymphocyte surface morphology ranging from smooth but slightly irregular (Fig. 3), relatively smooth with small stub-like projections (Fig. 4), relatively smooth with a moderate number of surface digitations (Fig. 5), to intermediate surface morphology with a relatively large number of surface microvilli (Fig. 6). × 14,000.

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1–2%, possessed both markers. From the comparison of the above data concerning percentages of B and T cells and the SEM results that showed that approximately 20% of the cells were of the villous type with the smaller relatively smooth cells making up approximately 80%, it appeared likely that the villous cells corresponded to B cells and the relatively smooth cells corresponded to T cells. This interpretation received further support from the study of the following samples.

One normal individual (Table I) was known to have a consistently high level of B cells among his PBL. Repeated evaluation using immunologic markers showed 38–43% B cells and 58–69% T cells. SEM examination also consistently showed a higher percentage of villous cells than the other normal peripheral blood samples (40.5–56.2% B villous cells and 43.8–59.5% T relatively smooth cells) using the surface morphology criteria outlined above.

TABLE I

Correlation between Surface Markers of B and T Cells and Surface Morphology for Normal Peripheral Blood Lymphocytes and Preparations Enriched in B Cells

	Sheep cell rosettes	Relatively smooth cells	Ig staining	Villous cells
	%	%	%	%
Normal PBL				
Subject 1	78	74	22	26
Subject 2*	60	52	40	48
PBL enriched in B cells				
Subject 1	14	35	87	65
Subject 2*	20	28	80	72

* Individual with low T cell rosettes and high Ig staining.

Recently it has been possible to enrich the B cell population from normal peripheral blood by centrifugation after rosetting of the T cells. This was carried out on several of the normal peripheral blood cell preparations. After one such experiment the B cells were enriched so that they comprised 87% of the total cells and the T cells depleted to a corresponding degree. SEM analysis on this preparation showed a higher percentage of the villous cells (Table I).

Human thymocytes: 90–100% of the thymic cells formed SRBC rosettes and 0–2% were found to show surface immunoglobulins. SEM indicated that 70% of the cells with an average diameter of 4.8 μm (range 4.3–5.3 μm) were almost entirely smooth with some degree of surface irregularity showing up to 10 digitations per exposed cell surface (Fig. 7). These digitations were quite similar to those described above for the presumed T lymphocytes and were either stub-like (generally between 50 and 75 nm broad and some from 100 to 425 nm) or short microvilli (generally 210–500 nm long and rarely between 785 nm

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and 1.21 μm). About 30% of the cells had more than 10 digitations per exposed cell surface (range 10–40, average 28), half of these being stub-like in appearance and the remainder microvilli. An exceptional cell with multiple surface microvilli resembling the presumed B cells of the peripheral blood was seen in these micrographs.

Cultured Lymphoblastoid Cell Lines.—

MOLT-4 cell line: Evidence has been presented that this is a human T cell line (26). This was confirmed in the present studies and 65% of these cells formed SRBC rosettes and no surface Ig was detectable. Almost all cells examined by SEM were of the smooth cell type. The majority of cells were smooth, with a slightly irregular surface (Fig. 8), but some cells had from 4 to 34 (average 14) digitations per exposed surface and, when present, these were generally stub-like digitations or microvilli.

SK-LN, SK-L7, and 8866 lymphoid cell lines: These were all identified as primarily of B cell origin and had surface immunoglobulins and aggregate receptors with very low or absent rosette-forming cells. From 75 to 85% of the cells in these four cultured cell lines were similar to the presumed PBL B cells (Fig. 9) with long surface microvilli (up to 250 per exposed cell surface) of varying length (up to 2.7 μm) and from 120 to 350 nm in breadth. Cells of the 8866 cell line often had a striking surface morphology with multiple microvilli and filopodia that were generally erect, sharply defined, and some, in contrast to normal B PBL, reached up to 2.8 μm in length (Fig. 10).

Chronic Lymphocytic Leukemia (CLL).—The leukemic cells in five of the six cases were demonstrated to be of the B cell type and showed Ig staining and Fc receptors by the aggregate technique. The latter procedure has been demonstrated to be the more reliable one for CLL lymphocytes in previous studies (14, 32). Table II shows that over 90% of the cells in each of the five cases had the Fc receptor and low levels of rosetting cells. By SEM 85–99% of the leukemic cells from these patients had the highly villous surface morphology (Fig. 11) with from 55 to 250 microvilli per exposed surface (110–600 nm broad and up to 1.5 μm long). In the sixth patient, case De with a very high cell count (120,000/mm^3), a mixed population of leukemic cells as identified by immunologic analysis. This was an unusual CLL case and will be described in detail separately. In this case 60% of the cells formed SRBC rosettes and 42% had surface immunoglobulins, while by SEM 55% of cells were of B cell type and 45% of T cell morphology (Figs. 12 and 13). An additional case, which was not included in the above description because of chemotherapy, showed considerable numbers of atypical cells that were difficult to classify by SEM; these were B cells by immunological criteria.

DISCUSSION

Until recently most workers have prepared human blood cells and lymphocytes for SEM by air drying, a procedure which is known to cause distortion

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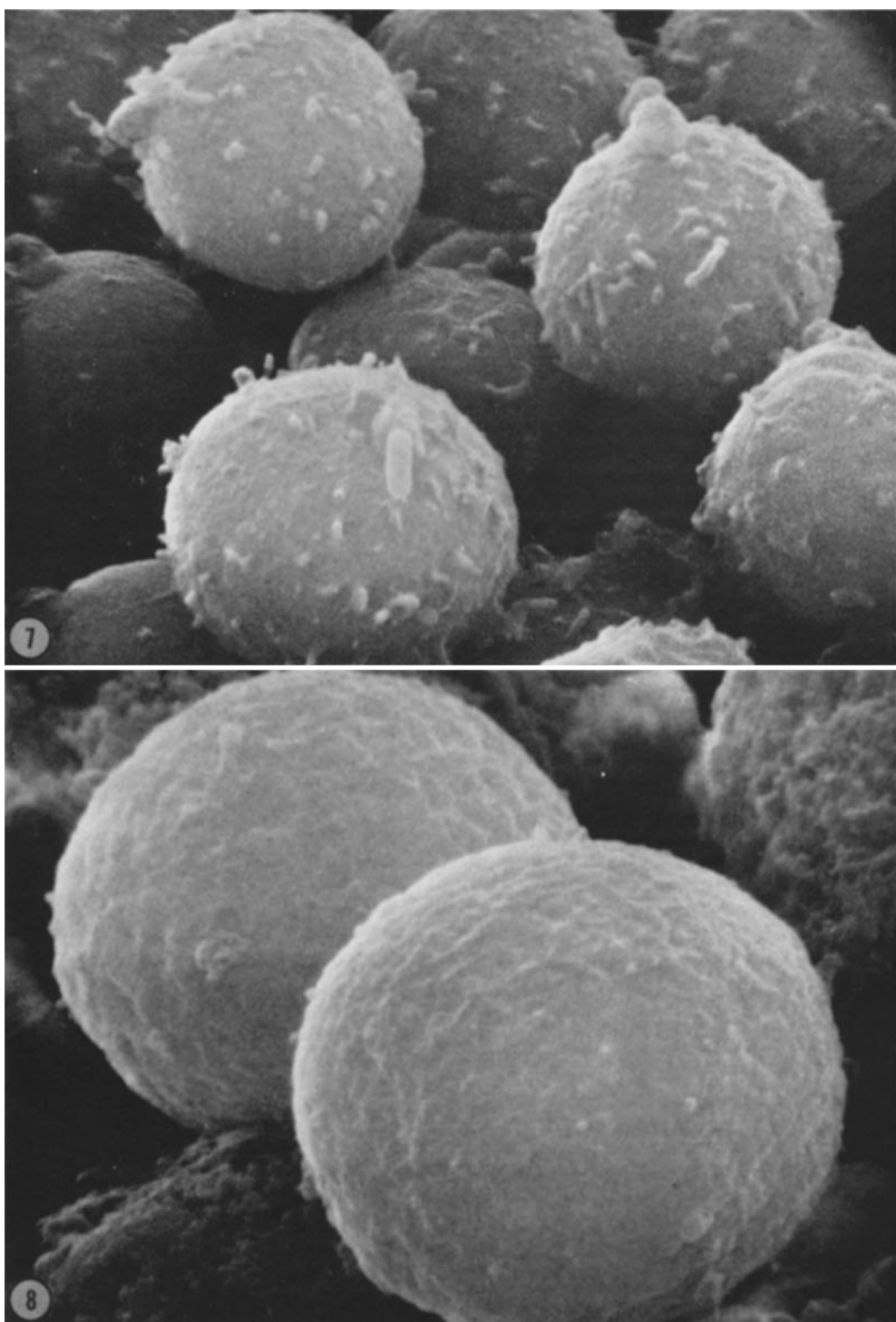

FIG. 7. Human thymic cells with generally smooth T cell surface morphology and a moderate number of surface digitations. × 10,000.

FIG. 8. Cultured lymphoid cells (MOLT-4) with typical T lymphocyte surface morphology. × 11,500.

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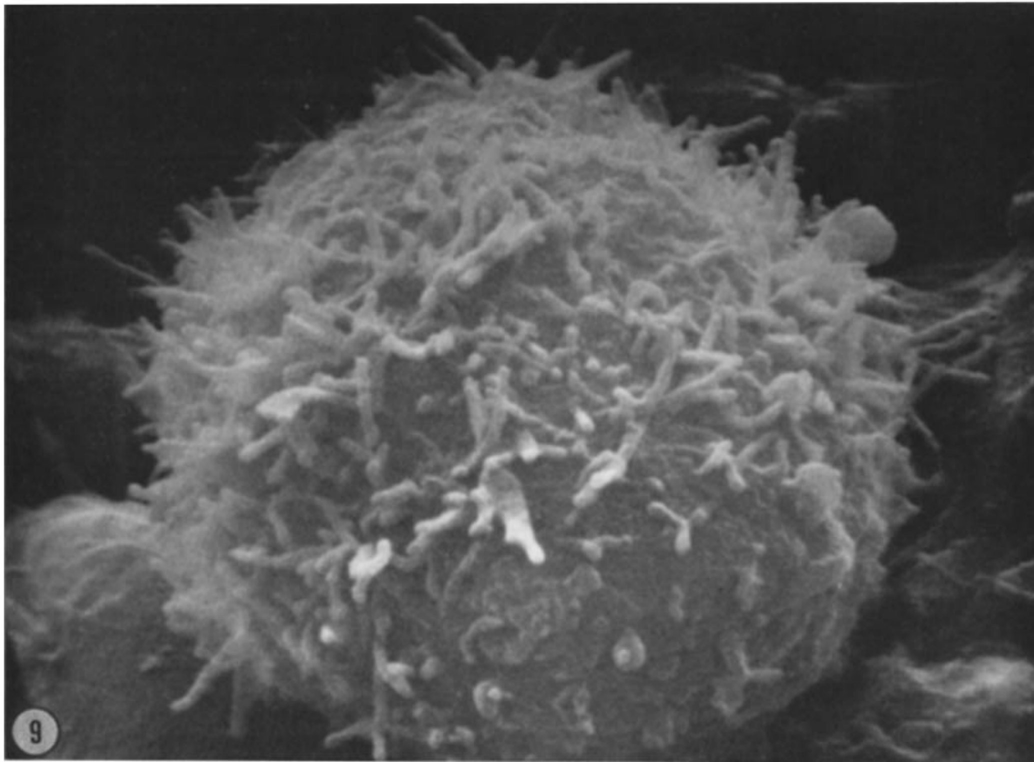

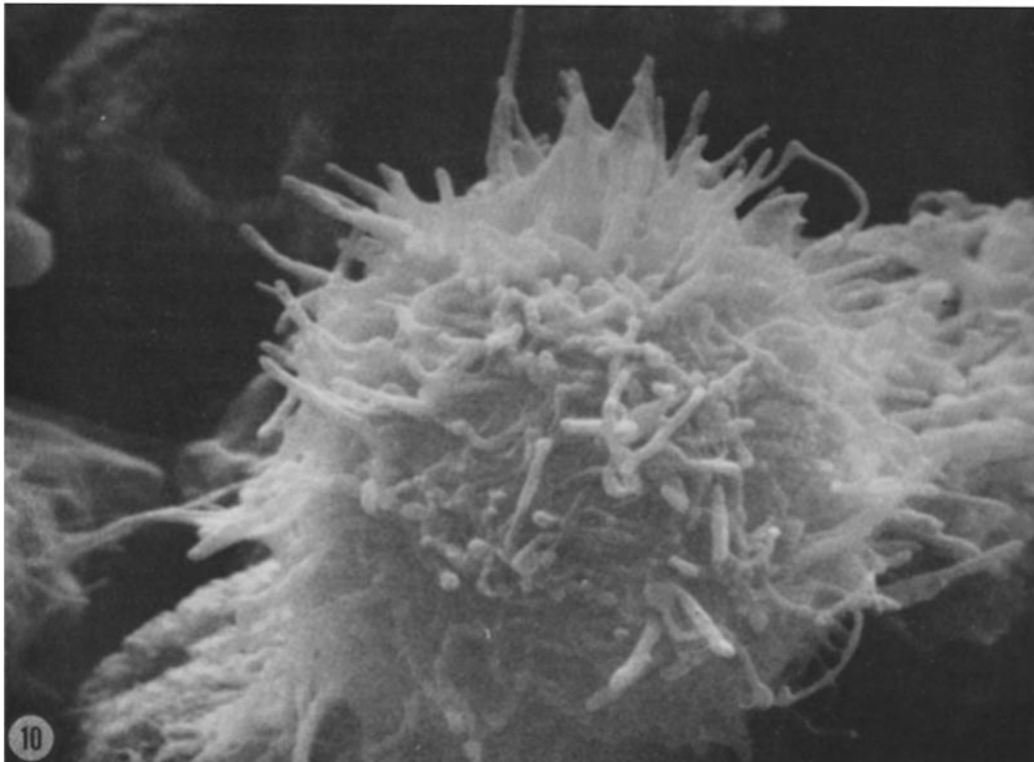

FIG. 9. Surface of SK-L7 lymphoid cell covered with a large number of microvilli, similar to, but generally longer than, those of the B lymphocyte. × 9,500.

FIG. 10. Cultured 8866 lymphoid cell, identified as B type with multiple long and erect microvilli. × 9,000.

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TABLE II
Correlation between Cell Surface Markers and Cell Morphology for the Leukemic Cells of Patients with CLL

Patients	Aggregate uptake	Villous cells	Sheep cell rosettes	Relatively smooth cells
	%	%	%	%
And.	98	99	7	1
Bot.	94	92	10	8
Mil.	99	85	0	15
Ber.	98	95	4	5
Lif.	95	91	4	9
De.	42	55	60	45

of the cell surface (33–36). However, since the introduction of critical point drying and freeze drying in SEM, and its application to the study of mammalian cells (22, 23, 37), much better preservation of surface detail has been obtained. In the present study critical point drying was used and enabled one to distinguish different lymphocyte types on the basis of their surface architecture. As in other studies (22, 37), this drying procedure was associated with a mild degree of shrinkage of the cells but surface detail was excellently preserved.

In this study, about 20% of normal PBL presented a complex surface architecture with an average of 150 microvilli, of varying length, covering almost the entire exposed surface. These cells were slightly larger than the majority (80%) of PBL, which presented a generally smooth, slightly irregular surface with or without a moderate number of surface digitations that, when present, did not cover the entire exposed surface. When these relatively smooth cells were examined under much higher magnifications, a small proportion of them, while still maintaining their general surface morphology, appeared to have a slightly more complex surface architecture, showing an average of about 50 digitations per exposed cell surface. These cells were difficult to classify and constituted a small group of lymphocytes with intermediate surface morphology.

A wide variety of immunologic data have shown that the majority of PBL in humans are T cells (65–85%) and that from 15 to 35% are B cells (31, 38, 39). In the present study two markers were utilized to demonstrate B cells, surface Ig and uptake of aggregated γ-globulin. The sheep cell rosette test was utilized as the primary marker of the T cells. Previous studies have demonstrated that the two B cell markers give very similar results and detect separate cells from those determined by the sheep cell rosette test (11, 14); the combination of the assays give values close to 100% for the cells of normal peripheral blood. When comparisons were made between these assays and the SEM for different blood samples, it became apparent that the B cells were those with the complex villous surface architecture while T lymphocytes corresponded to those with a relatively smooth or slightly irregular surface architecture. This

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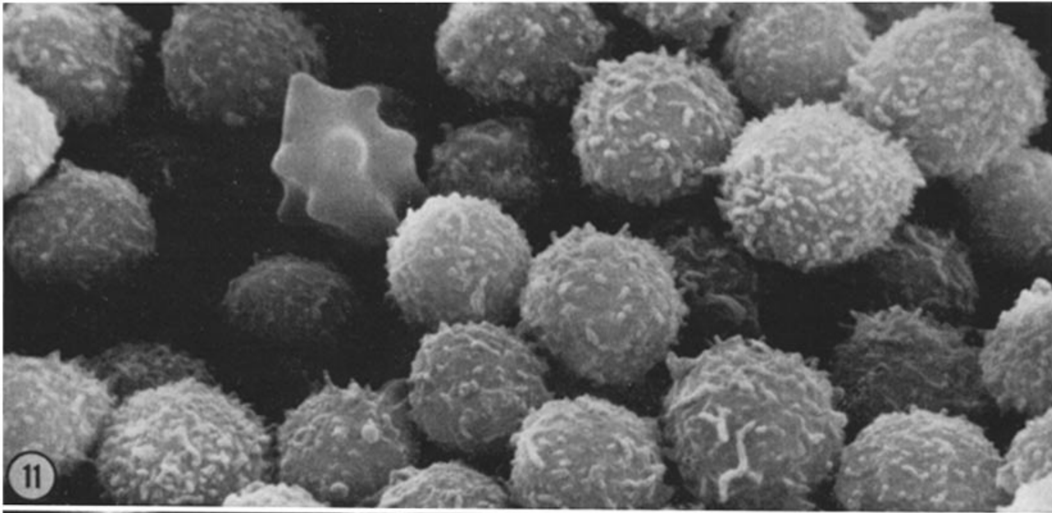

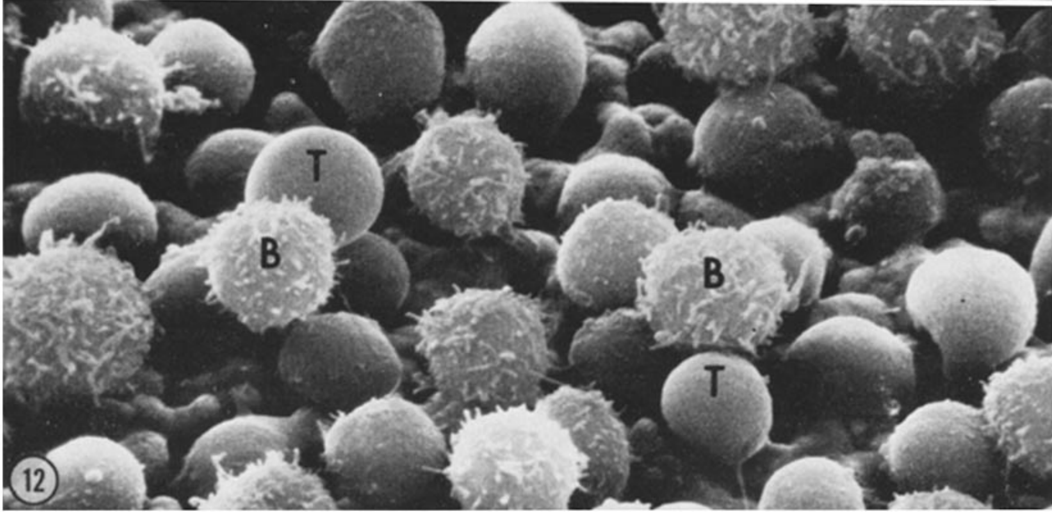

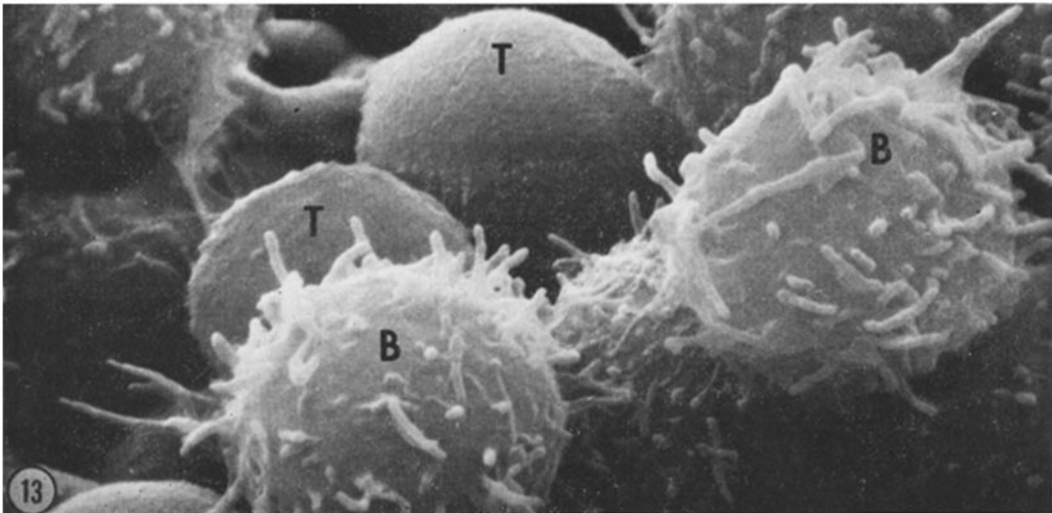

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was also evident from experiments where the B cells were obtained in a concentrated form after separation from the rosetted T cells; the cells with the complex villous surface were also concentrated.

Detailed examination of the smoother cells that corresponded to the rosette-forming T cells indicated that at least half of the cells may have a moderate number of surface microvilli or stub-like digitations. Another small group of cells with intermediate surface morphology, showing more surface digitations than the average T cell, were still believed to be T related because of their smaller size, their general surface architecture, and the moderate number of surface digitations many of which were still stub-like in form. These cells may well constitute a small subpopulation of T cells with a somewhat more complex surface architecture; however, it is difficult to exclude the possibility that they are in fact a group of smaller B cells with slightly less digitations and microvilli than the average B cell.

Further evidence for these relationships was obtained from studies of various lymphoid cell lines. One of these was identified as a T cell line. Ig staining and aggregate uptake were absent but the vast majority of cells formed rosettes. These cells were found to have only T cell surface morphology by SEM. Almost all cells were smooth and only a few had a moderate number of surface projections and folds. In addition human thymic cells when examined by SEM were similar in size and surface architecture to these T cells and those seen in the peripheral blood. Among the thymocytes a spectrum of surface architecture was seen ranging from cells with a generally smooth slightly irregular surface without digitations to those showing stub-like digitations or some short microvilli. It might well be that the varying surface morphology of some of the T lymphocytes and thymic cells correspond to lymphoid subpopulations of T cells at different stages of differentiation.

The examined cases of chronic lymphocytic leukemia (CLL) also provided more support for the above relationships and particularly for the identification of B lymphocyte surface morphology. It is generally agreed that this type of lymphocytic leukemia is primarily a malignant deviation of B cells (32, 39–41) although one case of CLL of T cell origin has been observed (14). The cells in five of our six cases were clearly B cells by immunologic methods and all had predominantly one cell type of quite uniform size, which like the normal B PBL, had a complex villous surface architecture. One other case was established as of mixed T and B leukemic cells by immunologic markers and clearly showed the presence of the mixture of B and T type lymphocytes by SEM. This case

Fig. 11. Leukemic lymphocytes from a buffy coat of a patient with B-type CLL, showing uniform pattern of B cell morphology. × 4,000.

Fig. 12. Leukemic lymphocytes from a patient with mixed B and T-type CLL, showing B and T cells in almost equal proportions. × 3,500.

Fig. 13. Higher magnification of Fig. 12 showing B and T types of leukemic lymphocytes. × 8,600.

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was of particular interest, demonstrating the presence of T and B lymphocytes with their markedly different surface architecture, in almost equal numbers.

In this study normal B PBL and leukemic or cultured lymphocytes identified as B cells generally had a similar surface pattern; however, the cultured and leukemic lymphocytes appeared to have more microvilli that were on the average longer than those of normal B PBL.

A large accumulation of information is available that serves to distinguish B and T lymphocytes on the basis of their different functions in the immune response and by their different membrane properties and surface receptors as demonstrated by a variety of morphologic methods including immunofluorescence, immunoelectron microscopy, and autoradiography (15–18). However despite these striking differences, no major morphologic distinctions have as yet been found by routine light and transmission electron microscope studies (14, 19–21), and distinguishing between B and T lymphocytes has depended, almost entirely, on the use of other methods. Some authors have described morphologic differences between what they called inactive lymphocytes and intermediate cells by electron microscopy (19), but others were unable, even after specific identification of B and T lymphocytes by rosette formation, to distinguish major morphologic differences between the two types of cells by electron microscopy (14, 21). Recently Matter et al. (42) have described pathways of differentiation in murine B and T lymphocytes by electron microscopy after identification of the cells with surface markers. It should be stressed that even by SEM, samples of different populations of lymphocytes could not be distinguished when prepared by air drying (37).

The results of the present study indicate that SEM of critical point dried specimens allows distinction between B and T lymphocytes, on the basis of their surface architecture, with relative ease in most cases. The varying surface morphology of T cells and particularly for PBL presents some problems in classification but these may correspond to different stages of differentiation and be indicative of the presence of subpopulations of lymphocytes. Studies, presently in progress on SRBC rosettes, stimulated T cells, and comparison with murine lymphocytes may well provide further important information in this respect. Others have found changes in the surface morphology of clutured cells in response to progression through the cell cycle and intercellular contact (43), thus it will also be of interest to determine what effects cell cycle, differentiation, cell confluence, and the number of circulating cells have on the surface architecture of leukemic and cultured cells.

SUMMARY

In this study a variety of human lymphocytes of known B or T cell type, obtained from multiple sources, were prepared for scanning electron microscopy (SEM) by the critical point drying method. Distinction between normal B and T lymphocytes was relatively easy in most instances, on the basis of their surface

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architecture. Using immunological methods, between 20 and 30% of normal peripheral blood lymphocytes (PBL) were identified as B cells and from 69 to 82% as T cells. SEM results showed that 20% of the PBL had a complex villous surface and approximately 80% of cells were smaller and had a relatively smooth surface. Comparison of the above data and enrichment of B cells from PBL, by centrifugation after T cell rosettes had formed, indicated that the "villous" cells were B lymphocytes and the "relatively smooth" cells were T lymphocytes. T cells obtained from two human thymuses were also of the generally smooth cell type.

Further evidence for the distinction of B and T lymphocytes, on the basis of surface morphology, was obtained from the examination of cultured lymphoid cell lines of known B or T cell derivation. Cells from cases of chronic lymphocytic leukemia also provided support for the above interpretations. Five of six untreated cases were clearly of B cell type by immunologic and SEM criteria. One unusual case showed the presence of T and B lymphocytes in almost equal numbers by SEM and a mixture of B and T cells by immunologic markers. An additional case that had received chemotherapy showed numerous atypical cells that were difficult to classify by SEM.

Detailed examination of the smoother T cells showed that at least half of them had a moderate number of surface digitations and a small proportion had an intermediate surface morphology with a relatively large number of surface digitations. The latter presented difficulties in classification and may correspond to different stages of differentiation and represent subpopulations of lymphocytes.

The distinction between human B and T lymphocytes on the basis of their surface architecture can be made by SEM of critical point dried samples, with relative ease in most but not all instances. The effects of stimulation, cell cycle, differentiation, intercellular contact, and density of cell population, on the surface architecture of lymphoid cells, remain to be determined.

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REFERENCES

1. Good, R. A. 1970. Progress towards a cellular engineering. *J. Am. Med. Assoc.* **214:**1389.
2. Raff, M. C. 1971. Surface antigenic markers for distinguishing T and B lymphocytes in mice. *Transplant. Rev.* **6:**52.
3. Wioland, M., D. Sabolovic, and D. Burg. 1972. Electrophoretic mobilities of T and B cells. *Nat. New Biol.* **237:**274.

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4. Nordling, S., L. C. Andersson, and P. Häyry. 1972. Separation of T and B lymphocytes by preparative cells electrophoresis. *Eur. J. Immunol.* **2**:405.
5. Boyse, E. A., L. J. Old, and E. Stockert. 1968. An approach to the mapping of antigens on the cell surface. *Proc. Natl. Acad. Sci. U.S.A.* **60**:886.
6. Schlesinger, M., and I. Yron. 1970. Serologic demonstration of a thymus-dependent population of lymph nodes. *J. Immunol.* **104**:798.
7. Takahashi, T., L. J. Old, R. K. McIntire, and E. A. Boyse. 1971. Immunoglobulin and other surface antigens of cells of the immune system. *J. Exp. Med.* **134**:815.
8. Douglas, S. D., R. M. Kamin, W. E. Davis, and H. H. Fudenberg. 1969. Biochemical and morphologic aspects of phytomitogens. *In* Proceedings of Third Annual Leucocyte Culture Conference. W. O. Rieke, editor. Appleton-Century-Crofts, New York. 607.
9. Andersson, J., O. Sjöberg, and G. Moller. 1972. Mitogens as probes for immunocyte activation and cellular cooperation. *Transplant. Rev.* **11**:131.
10. Rabellino, E., S. Colon, H. M. Grey, and R. E. Unanue. 1971. Immunoglobulins on the surface of lymphocytes. I. Distribution and quantitation. *J. Exp. Med.* **133**:156.
11. Dickler, H. B., and H. G. Kunkel. 1972. Interaction of aggregated γ-globulin with B lymphocytes. *J. Exp. Med.* **136**:191.
12. Bianco, C., R. Patrick, and V. J. Nussenzweig. 1970. A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. *J. Exp. Med.* **132**:702.
13. Wybran, J. H., H. H. Fudenberg, and M. H. Sleisenger. 1971. Rosette formation. A test for cellular immunity. *Clin. Res.* **19**:568.
14. Bentwich, Z., S. D. Douglas, F. P. Siegal, and H. G. Kunkel. 1973. Human lymphocyte-sheep erythrocyte rosette formation. Some characteristics of the interaction. *Clin. Immunol. Immunopathol.* In press.
15. Aoki, T., U. Hammerling, E. de Harven, E. A. Boyse, and L. J. Old. 1969. Antigenic structure of cell surfaces. An immunoferritin study of the occurrence of topography of H-2, θ, and TL alloantigens on mouse cells. *J. Exp. Med.* **130**:979.
16. Hammerling, U., T. Aoki, H. A. Wood, L. J. Old, E. A. Boyse, and E. de Harven. 1969. New visual markers of antibody for electron microscopy. *Nature (Lond.).* **223**:1158.
17. Gonatas, N. K., J. C. Antoine, A. Stieber, and S. Avrameas. 1972. Surface immunoglobulins of thymus and lymph node cells demonstrated by the peroxidase coupling technique. *Lab. Invest.* **26**:253.
18. Perkins, W. D., M. J. Karnovsky, and E. R. Unanue. 1972. An ultrastructural study of lymphocytes with surface-bound immunoglobulin. *J. Exp. Med.* **135**:267.
19. Zucker-Franklin, D. 1969. The ultrastructure of lymphocytes. *Semin. Hematol.* **6**:1.
20. Douglas, S. D. 1971. Human lymphocyte growth in vitro: morphologic, biochemical and immunologic significance. *Int. Rev. Exp. Pathol.* **10**:41.
21. Chen, L. T., A. Eden, V. Nussenzweig, and L. Weiss. 1972. Electron microscopic study of the lymphocytes capable of binding antigen-antibody-complement complexes. *Cell. Immunol.* **4**:279.

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22. Boyde, A., R. A. Weiss, and P. Vesely. 1972. Scanning electron microscopy of cells in culture. *Exp. Cell Res.* **71**:313.

23. Porter, K. R., D. Kelley, and P. M. Andrews. 1972. The preparation of cultured cells and soft tissues for scanning electron microscopy. *In* Proceedings of Fifth Annual Stereoscan Colloquium, April. Kent Cambridge Scientific, Inc., Morton Grove, Ill. 1.

24. Todo, A., A. Strife, J. Fried, and B. D. Clarkson. 1971. Proliferative kinetics of human hematopoietic cells during different growth phases in vitro. *Cancer Res.* **31**:1330.

25. Lerner, R. A., P. J. McConahey, I. Jansen, and F. J. Dixon. 1972. Synthesis of plasma membrane-associated and secretory immunoglobulin in diploid lymphocytes. *J. Exp. Med.* **135**:136.

26. Minowada, J., T. Ohnuma, and G. E. Moore. 1972. Rosette forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus derived lymphocytes. *J. Natl. Cancer Inst.* **49**:891.

27. Boyum, A. 1968. Separation of leukocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest. Suppl.* **21**:97.

28. Grothaus, E. A., E. J. Rauckman, and D. B. Amos. 1971. Conditions affecting the performance of the lymphocyte toxicity test. *Transplantation.* **11**:145.

29. de Harven, E., N. Lampen, T. Sato, and C. Friend. 1973. Scanning electron microscopy of cells infected with a murine leukemia virus. *Virology.* **51**:240.

30. Anderson, T. F. 1951. Techniques for preservation of 3-dimensional structure in preparing specimens for electron microscopy. *Trans. N. Y. Acad. Sci.* **13**:130.

31. Siegal, F. B., B. Pernis, and H. G. Kunkel. 1971. Lymphocytes in human immunodeficiency states: a study of membrane-associated immunoglobulins. *Eur. J. Immunol.* **1**:482.

32. Dickler, H. B., F. P. Siegal, Z. H. Bentwich, and H. G. Kunkel. 1973. Lymphocyte binding of aggregated IgG and surface Ig staining in chronic lymphocytic leukemia. *Clin. Exp. Immunol.* In press.

33. Bessis, M., and A. De Boisfleury. 1971. Les mouvements des leucocytes étudiés au microscopic electronique a balayage. *Nouv. Rev. Fr. Hematol.* **11**:377.

34. Holt, P. J. L., S. G. Pal, D. Catovsky, and S. M. Lewis. 1972. Surface structure of normal and leukemic lymphocytes. I. Effect of mitogens. *Clin. Exp. Immunol.* **10**:555.

35. Gormley, I. P., and A. Ross. 1972. The morphology of phytohaemagglutinin (PHA)-stimulated lymphocytes and permanent cell lines seen by the scanning electron microscope. *Eur. J. Cancer.* **8**:491.

36. Gudat, F. G., and W. Villiger. 1973. A scanning and transmission electron microscope study of antigen-binding sites on rosette-forming cells. *J. Exp. Med.* **137**:483.

37. Polliack, A., N. Lampen, and E. de Harven. 1973. Comparison of air drying and critical point drying procedures for the study of human blood cells by scanning electron microscopy. *In* Proceedings of 6th Annual Scanning Electron Microscopy Symposium, Chicago. IIT Research Institute, Chicago, Ill. 529.

38. Fröland, S. S., J. B. Natvig, and P. Berdal. 1971. Surface-bound immunoglobulin as a marker of B lymphocytes in man. *Nat. New Biol.* **234**:251.

39. Wilson, J. D., and G. J. V. Nossal. 1971. Identification of human T and B lympho-

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cytes in normal peripheral blood and in chronic lymphocytic leukemia. *Lancet.* **2:**788.

40. Preud'homme, J. L., M. Klein, P. Verroust, and M. Seligmann. 1971. Immunoglobulines monoclonales de membrane dans les leucémies lymphoides chroniques. *Rev. Eur. Etud. Clin. Biol.* **16:**1025.
41. Ross, G. D., E. M. Rabellino, M. J. Polley, and H. M. Grey. 1973. Combined studies of complement receptor and surface immunoglobulin bearing cells and sheep erythrocyte rosette-forming cells in normal and leukemic human lymphocytes. *J. Clin. Invest.* **52:**377.
42. Matter, A., B. Lisowska-Bernstein, J. E. Ryser, J. P. Lamelin, and P. Vassali. 1972. Mouse thymus-independent and thymus-derived lymphoid cells. II. Ultrastructural studies. *J. Exp. Med.* **136:**1008.
43. Rubin, R. W., L. P. Everhart, K. R. Porter, D. M. Prescott, and J. Frye. 1972. Changes in form and surface morphology of CHO cells in response to progression through the cell cycle and intercellular contact. *J. Cell Biol.* **55:**222a. (Abstr.)

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