

THE SURFACE MORPHOLOGY OF HUMAN B LYMPHOCYTES AS REVEALED BY IMMUNOELECTRON MICROSCOPY*

By F. REYES, J. L. LEJONC, M. F. GOURDIN, P. MANNONI, AND B. DREYFUS

(From the Unité de Recherches sur les Anémies U91, Institut National de la Santé et de la Recherche Médicale, and the Centre Départemental de Transfusion Sanguine du Val de Marne, CHU Henri-Mondor, 94010 Créteil, France)

Substantial interest in morphological studies of lymphocytes has been generated by the recognition of two functionally distinct populations, i.e. thymus-derived (T) and bone marrow or bursa-derived (B) lymphocytes. Basic findings were obtained from immunofluorescence studies which visualized surface immunoglobulins (sIg)¹ on B lymphocytes (1, 2). This method however does not allow a precise morphological identification of the involved cells. On the other hand immunoelectron microscopic methods have appeared which allow the simultaneous study of both surface components and fine structure of the cell. To date, however, no striking morphological differences have been reported by these methods between immunoglobulin-bearing lymphocytes and the others (3-6).

Since B and T lymphocytes have been distinguished basically by surface properties it was to be anticipated that differences of their membrane morphology could also be detected. Indeed, evidence has been recently given by scanning electron microscopy indicating that human B and T lymphocytes have a distinct surface morphology (7). In this work we demonstrate that the presence of sIg is associated with a peculiar surface architecture of normal human lymphocytes.

Material and Methods

Samples. The following samples were examined: blood buffy coat from eight normal individuals, seven untreated patients with chronic lymphocytic leukemia (CLL), and one with Sézary's syndrome. Bone marrow cells were also obtained from one patient with CLL and thymocytes from one normal individual.

Preparation of Specimens for Immunoelectron Microscopy. As a rule, cell suspensions were fixed before incubation with reagents. Such fixed cells could be kept without damage in buffer until the following steps.

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¹ *Abbreviations used in this paper:* anti-Ig, mixture of pure antibodies reacting with both human IgM and IgG; anti-IgM, anti-IgG, anti-IgA, pure monospecific anti-immunoglobulin antibodies; CLL, chronic lymphocytic leukemia; HBSS, Hanks' balanced salt solution; sIg, surface-bound immunoglobulins.

BLOOD CELLS. Fresh heparinized blood was withdrawn by venous puncture and erythrocytes allowed to sediment at room temperature for 1 h. The buffy coat was then aspirated in plasma, centrifugated at 200 g, and washed three times in a large volume of Hanks' balanced salt solution (HBSS) at room temperature. The cell pellet was then fixed by resuspending in glutaraldehyde (TAAB Laboratories, Emmer Green, Reading, England) 1.25% in phosphate-buffered saline (pH 7.4, 0.15 M) for 30 min and washed twice in the same buffer.

BONE MARROW CELLS. A suspension was obtained as previously described (8). Briefly, bone marrow particles are dissociated in HBSS, extensively washed, and fixed. As for blood leukocytes no attempt was made to isolate lymphocytes from the suspension since cells were to be recognized under the electron microscope.

THYMUS CELLS. Fresh human thymus was obtained from a 5-yr-old patient undergoing cardiac surgery. The tissue was teased in HBSS with fine forceps and cells concentrated by several centrifugations at 200 g, room temperature. After two additional washes the pellet was glutaraldehyde fixed, as above.

ANTI-IMMUNOGLOBULIN ANTIBODIES

ANTI-IGM AND ANTI-IGG. Antisera to human purified IgM and IgG were raised in rabbits, after a 2 mo immunization procedure (3). Normal and monoclonal IgG and IgM were purified from human sera according to methods already described (9, 10) and checked by immunoelectrophoresis and Ouchterlony assays. Purified IgM and IgG were also polymerized with glutaraldehyde according to Avrameas and Ternynck (11). These immunoadsorbents allowed the specific isolation of antibodies to IgM or IgG from rabbit hyperimmune sera. In addition these eluted antibodies were made monospecific for heavy chains by repeated cross-reactions with the appropriate polymer retaining antilight chain activity. Monospecificity was assessed by double diffusion and also after coupling to peroxidase, by staining of appropriate CLL lymphocytes with known sIg (see below). These monospecific anti-immunoglobulin antibodies will be referred as anti-IgM or anti-IgG. In a set of experiments an equal mixture of antibodies eluted from both IgM and IgG polymers was used, thus reacting with μ , γ , and light chains determinants; this mixture will be referred as anti-Ig.

ANTI-IGA. These antibodies were prepared as described elsewhere.² Briefly, sheep were immunized with human colostrum. Antiserum was cross-reacted with insolubilized human IgG fractions and IgA-deficient sera. Subsequent adsorption on insolubilized human sera with high polyclonal IgA content allowed the elution of pure monospecific anti-IgA antibodies.

NORMAL RABBIT γ -GLOBULINS. They were prepared by precipitation of pooled normal sera with 40% saturated ammonium sulphate.

CONJUGATION WITH HORSE RADISH PEROXIDASE. Rabbit anti-immunoglobulin antibodies and normal γ -globulins were covalently coupled to horseradish peroxidase (RZ3, Sigma Laboratories) by a two-step method in which the peroxidase is first mixed with glutaraldehyde and subsequently allowed to react with these reagents (12). Noncoupled peroxidase was then removed by precipitation of conjugated reagents with 50% ammonium sulphate. Sheep anti-IgA antibodies were coupled by the one-step method (13) and used without removal of free peroxidase.

IMMUNOCYTOCHEMICAL DETECTION OF sIg. Fixed cells were centrifugated and the cell pellet resuspended in phosphate-buffered saline (3 vol/100 vol). 1 ml of this 3% cell suspension was used in the following steps. Samples were exposed to the appropriate conjugated reagents (1 mg/ml) for 1 h at room temperature, washed twice in large volumes of cold buffer, fixed again for 15 min with 1.25% cold buffered glutaraldehyde and extensively washed.

Fixed anti-immunoglobulin-coated cells were incubated for 30 min in the usual mixture of diaminobenzidine and hydrogen peroxide in order to detect the peroxidase enzymatic activity (14). They were postfixated with 1% osmium tetroxide for 30 min, washed, and processed for dehydration in alcohols and embedding in Epon. Ultrathin sections were cut on a Reichert Ultramicrotome (C. Reichert, ISI, Paris, France) and examined with a Philips EM 300 (Philips Industry, Bobigny, France). Except when indicated in legends, sections were not counterstained with uranyl acetate or lead citrate since the resulting enhanced membrane contrast could obscure weak specific labeling.

CONTROLS. Since surface-bound IgM and IgG were detected by a direct method involving conju-

²Sapin, C., A. Contet, A. Massez, and Ph. Druet, 1974. Manuscript submitted for publication in *The Journal of Immunological Methods*.

gated hyperimmune rabbit antibodies, the following controls were used: (a) replacement of conjugated antibodies by conjugated normal rabbit γ -globulins (1 mg/ml) (b) exposure of CLL lymphocytes with known sIg to inappropriate conjugated monospecific anti-immunoglobulin antibodies (c) selective inhibition of the labeling as follows: cell exposure to nonconjugated antibody (1 mg/ml) for 1 h, brief glutaraldehyde fixation and exposure to the corresponding conjugated antibody. Since anti-IgA antibodies were used without removal of the noncoupled peroxidase, parallel controls were made in which fixed cells were exposed to an excess of horseradish peroxidase alone (3 mg/ml, in phosphate buffer).

Results

Normal Cells. A membrane labeling was found in 15–20% of blood lymphocytes, when exposed to the anti-Ig antibodies. A quantitative estimation of positive cells with respect to surface immunoglobulin classes was not under the scope of this study. However, IgM was obviously the main detected sIg in every normal sample, confirming preliminary results (15); IgG was only detected on a minority of lymphocytes and IgA-bearing cells were very uncommon in sections.

In every sample, the labeling was continuous along the membrane outlined by a dark dense reaction product. No cells were found with a “patchy” distribution of the labeling.

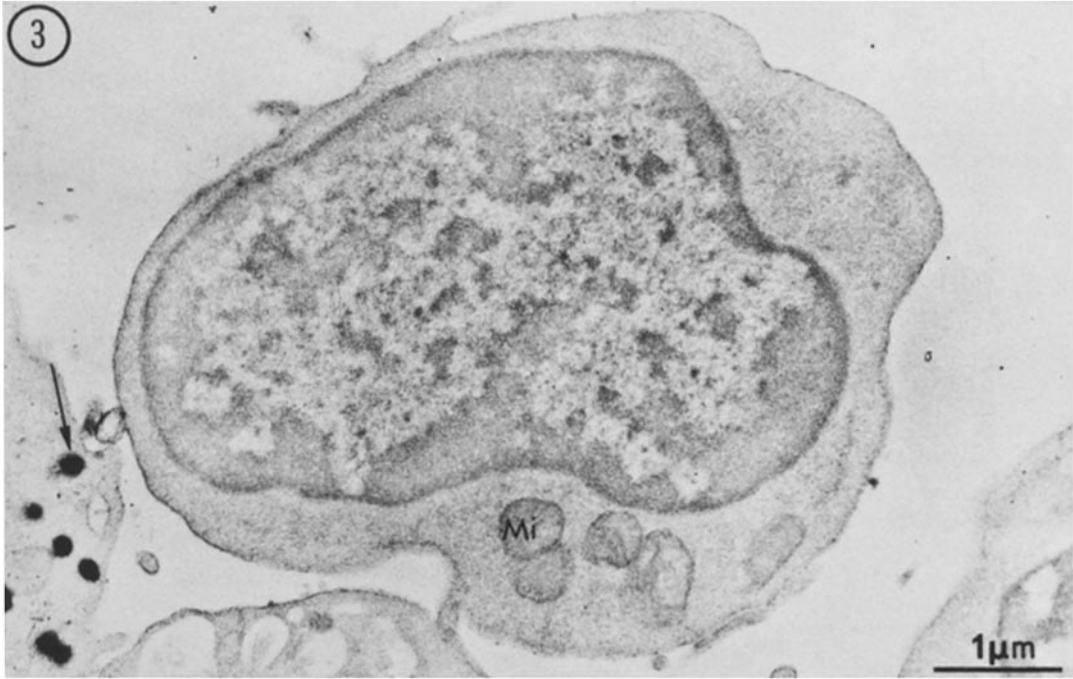
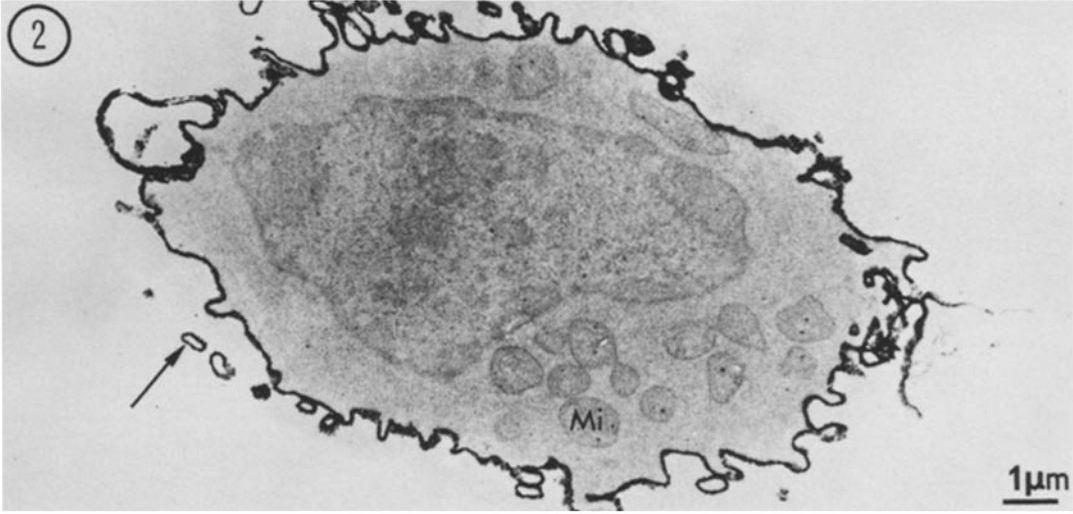
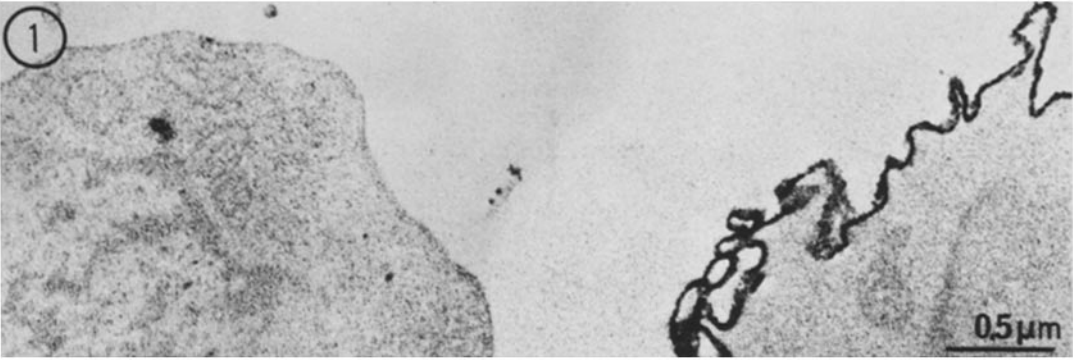
The most striking feature of blood-labeled lymphocytes was their villous surface; on the other hand nonlabeled lymphocytes had a smooth regular membrane (Figs. 1–4). Microvilli of labeled lymphocytes were numerous surrounding all the cell, varying in length according to the plane of section; cross-sections of these microvilli were frequent contiguous to or at some distance from the cell. In a few sections lymphocytes exhibited a polar concentration of the villous processes with parallel accumulation of the surface staining, the remaining membrane being weakly labeled. This feature, although not frequent, gave additional evidence for the relationship between villous surface and presence of sIg (Figs. 8, 9).

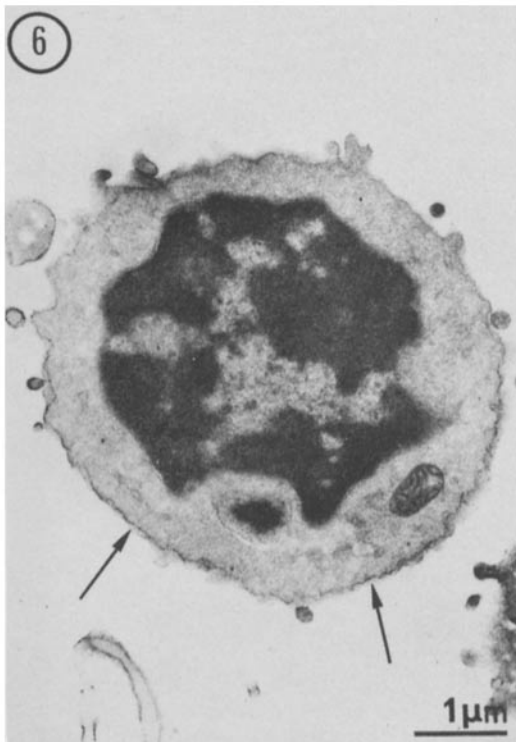
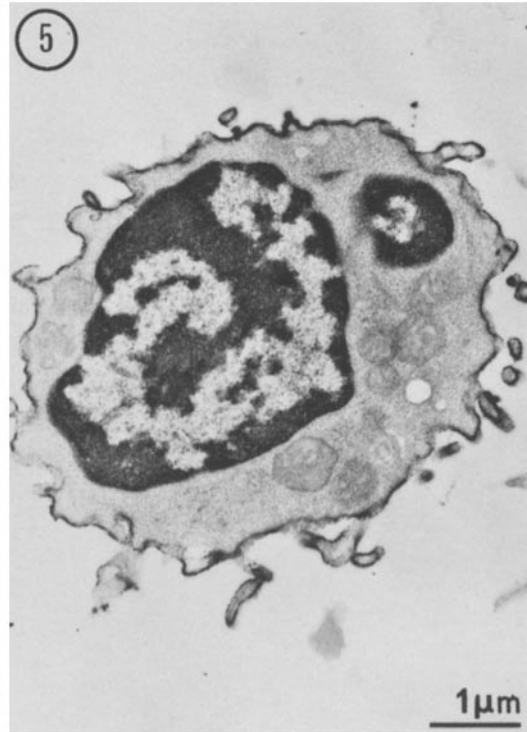
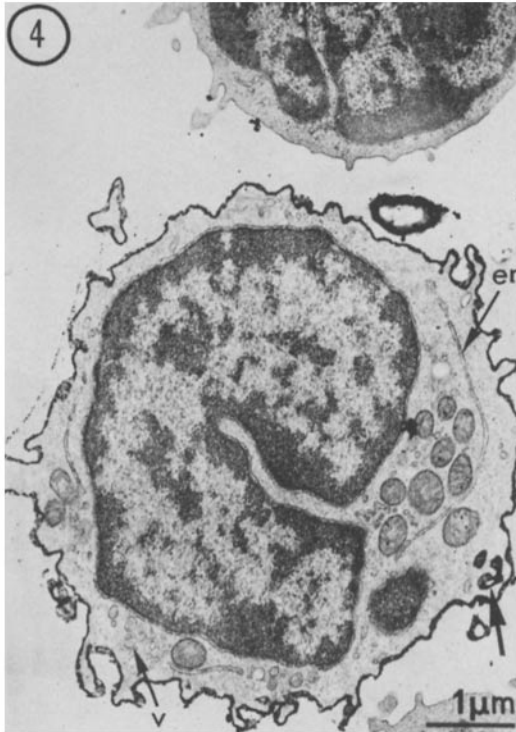
However, the morphological duality found in blood between villous and smooth cells was not absolute (Figs. 4–7). First, some nonlabeled “smooth” lymphocytes could exhibit a few short and spaced cytoplasmic digitations, but without affecting the regular shape of the membrane. Moreover, cells with a moderate surface staining also appeared to have a moderate number of microvilli when compared to the heavy labeled “hairy” lymphocytes. It is of interest to note that these intermediate forms of labeled lymphocytes were found in every sample of normal blood, when cells had been exposed to anti-Ig or anti-IgM antibodies (which allowed examination of a sufficient number of positive cells at this ultrastructural level). Thus, variations of the anti-immunoglobulin binding capacity paralleled variations of the number of microvilli as seen in sections.

FIG. 1. Buffy coat from normal blood. Fixed cells have been exposed to anti-Ig antibodies. Membranes are seen of one labeled villous and one nonlabeled smooth lymphocyte. sIg is revealed as an electron-dense product. $\times 30,000$.

FIG. 2. Large B lymphocyte from normal blood labeled by anti-IgM antibodies. This cell exhibits a villous surface. Arrow, cross-section of a microvilli. Mi, mitochondria. $\times 7,250$.

FIG. 3. Blood normal smooth lymphocyte. The membrane is not labeled. Its faint contrast results from osmium postfixation. Mi, mitochondria. A contiguous monocyte, with peroxidase-containing granules (arrow) is seen at lower left. $\times 17,000$.





Thymus cells exposed to anti-IgM or anti-Ig reagents were not labeled. They were numerous dark, small, and a few larger lymphocytes. All these cells had a very regular smooth membrane with on rare occasions a few and short spaced microvilli (Fig. 19).

Thus a clear distinction could be made between blood lymphocytes on the basis of their surface morphology. No major differences of intracellular organelles were observed, on usual ultrastructural grounds. However, for reasons described above, most sections were examined without counterstaining and this may account for an underestimation of some fine structural details.

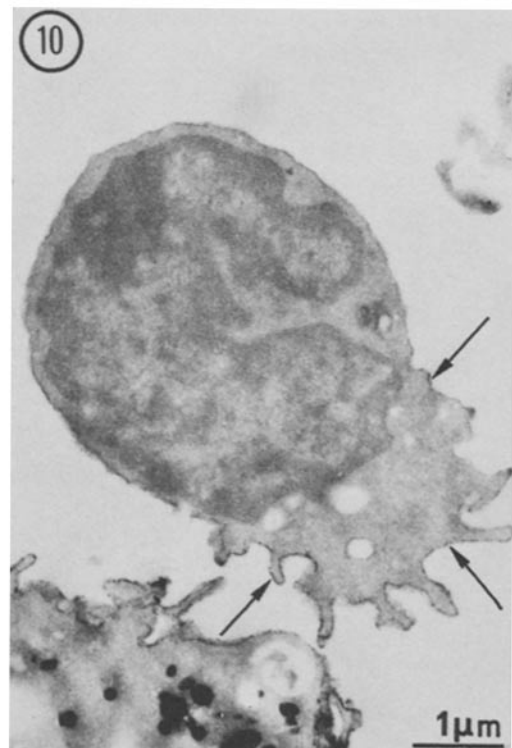
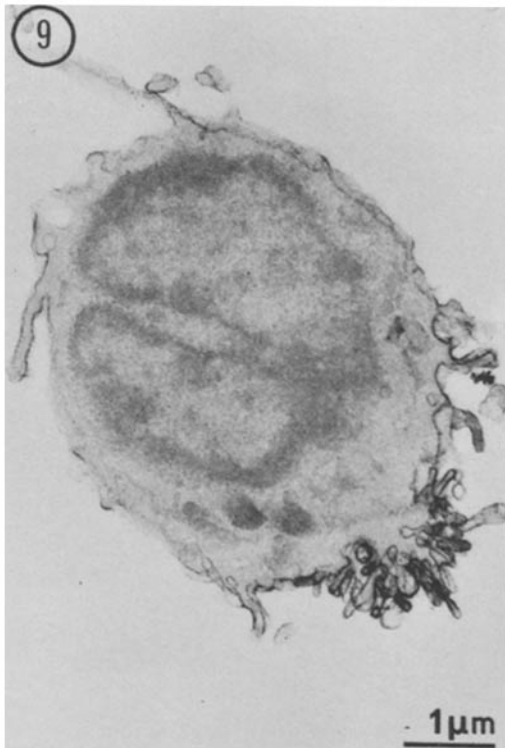
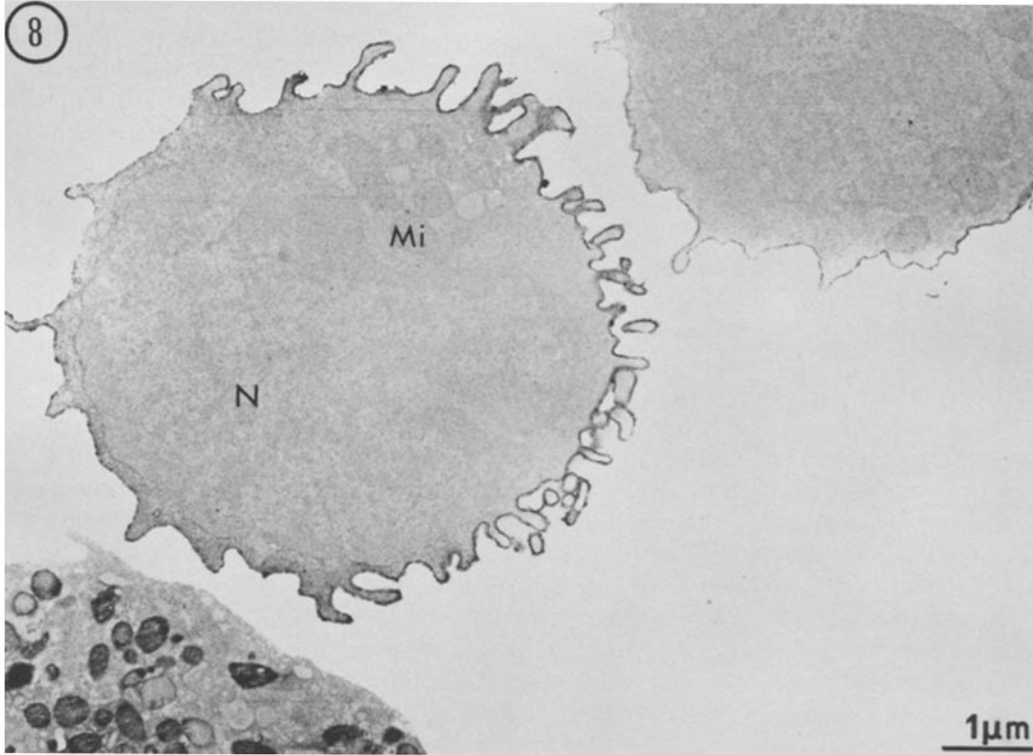
Cells from Lymphoproliferative Disorders. Five CLL samples were exposed to the monospecific anti-immunoglobulin antibodies: exclusive sIg was identified as IgM in four cases and IgG in one (bone marrow lymphocytes). sIg were detected in three additional CLL samples by exposure to anti-Ig antibodies.

Obvious variations of the membrane-staining intensity were found among CLL samples. Examination of large numbers of sections at low magnification made it clear that in several samples cells had a weak surface labeling, while they were more clearly stained in the remaining. In the latter sIg were detected on virtually all cells (Fig. 14). Moreover, variations of the staining intensity were also apparent among lymphocytes of a given sample, when labeled by monospecific antibodies (Fig. 15); these individual cell variations, which included some negative cells, were more obvious in specimens exhibiting a weak general labeling. As in normal blood, a general relationship was apparent between the surface of CLL lymphocytes and the detection of sIg, the more villous cells being found in heavily stained samples.

However, two CLL blood specimens with sIg identified as IgM comprised a definite amount of larger cells with ultrastructural features of "young" nucleus, i.e., with no or little heterochromatin and a large nucleolus. These cells were easily detected on routine blood smears and termed "lymphoblasts"; they accounted for 10% and 30% of blood lymphocytes. In sections they appeared to have a smooth membrane with few or no microvilli and had clearly detectable surface IgM (Figs. 16 and 17). This detection of an appreciable amount of labeled smooth lymphocytes was at variance with findings from normal blood.

In patient with Sézary's syndrome half of blood leukocytes were abnormal lymphoid cells, easily recognized by usual criteria among which convoluted nucleus is the most obvious (16). They were large cells with cytoplasm containing numerous lucent vesicles, multivesicular bodies and dense small lysosomes.

FIG. 4-7. Spectrum of normal blood lymphocytes, ranging from labeled (anti-Ig) villous to smooth nonlabeled cells. Sections have been counterstained with lead citrate. $\times 12,000$. Fig. 4 shows classical intracytoplasmic features of a resting lymphocyte, i.e. poorly developed endoplasmic reticulum (er), monoribosomes, numerous mitochondria and pinocytotic vesicles (v): nucleus is typical with clumps of dense heterochromatin. Arrow, surface invagination seen in tangential section and thus appearing as an intra-cellular label. Facing smooth lymphocyte is not labeled and has some short and spaced microvilli. Intermediate B lymphocytes have surfaces ranging from moderately villous and labeled (Fig. 5), to poorly villous and weakly labeled (Fig. 6). Fig. 7 is a typical small lymphocyte without developed endoplasmic reticulum and with diffuse monoribosomes; surface is free of detectable sIg and smooth although slightly irregular exhibiting rare villous processes.



On some occasions these abnormal lymphocytes had segments of villous membrane (Fig. 18). No surface labeling was found however after exposure to the various anti-immunoglobulin reagents in contrast to the very few villous typical lymphocytes present in this sample (Fig. 11).

Contaminating Cells. As stated above (see Materials and Methods) no attempt was made to separate lymphocytes from other leukocytes, in normal and patient samples. This procedure allowed the simultaneous observation of a few contaminating erythrocytes and of monocytes and granulocytes, which exhibit an endogenous peroxidase activity. In no sample labeled erythrocytes were found. The vast majority of granulocytes was also free of labeling (Figs. 8 and 11). However a few of them and a variable amount of monocytes were found to be labeled, mostly at the level of their membrane fingerlike projections (Figs. 10 and 12). This occurred whether the monocytes were contiguous or not to a positive-labeled lymphocyte. On the other hand, the membrane of contiguous monocytes could also be free of labeling (Fig. 13).

Controls. The specificity of lymphocyte surface labeling was ensured in several ways. Normal and CLL lymphocytes were free of surface staining after exposure to conjugated normal rabbit IgG; interestingly, contaminating leukocytes were also free of label. CLL lymphocytes which had proved to stain with one given monospecific anti-immunoglobulin reagent were devoid of labeling when exposed to the others. Finally, inhibition of labeling was demonstrated when carried out on normal (with anti-Ig antibodies) and CLL (with monospecific antibodies) lymphocytes.

The majority of normal and CLL fixed cells were found without surface staining when incubated with horseradish peroxidase along. In some sections however a thin and discontinuous dark precipitate could be found along the membrane (inset Fig. 15). This artefactual staining, easily distinguished from specific reaction, was never observed in experiments using conjugates which were devoid of free noncoupled peroxidase.

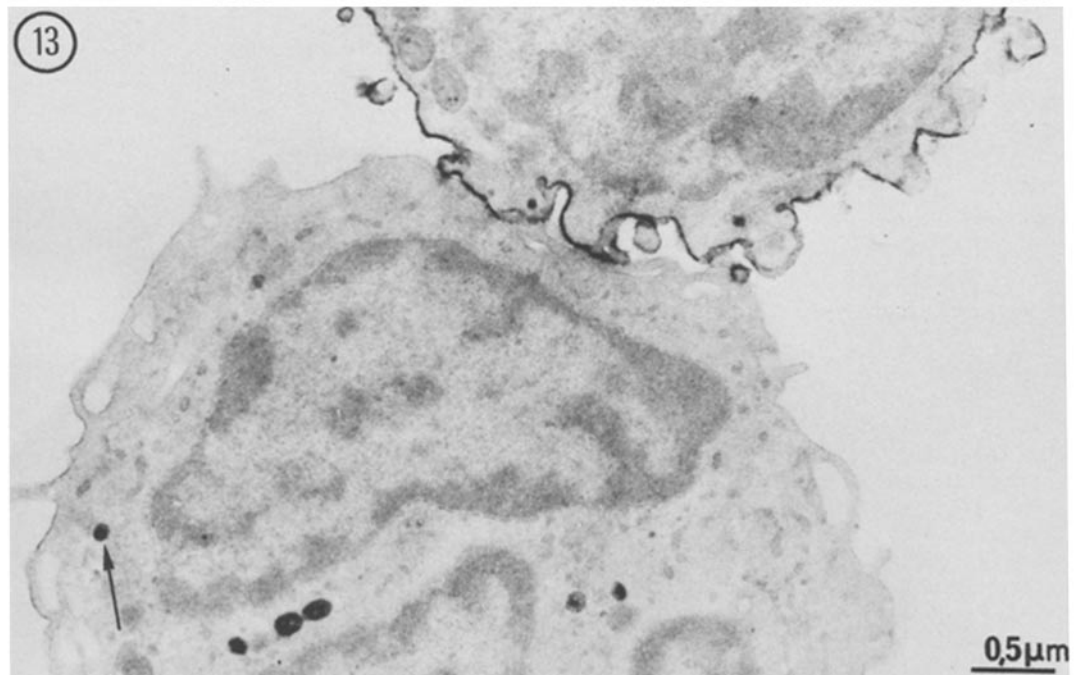
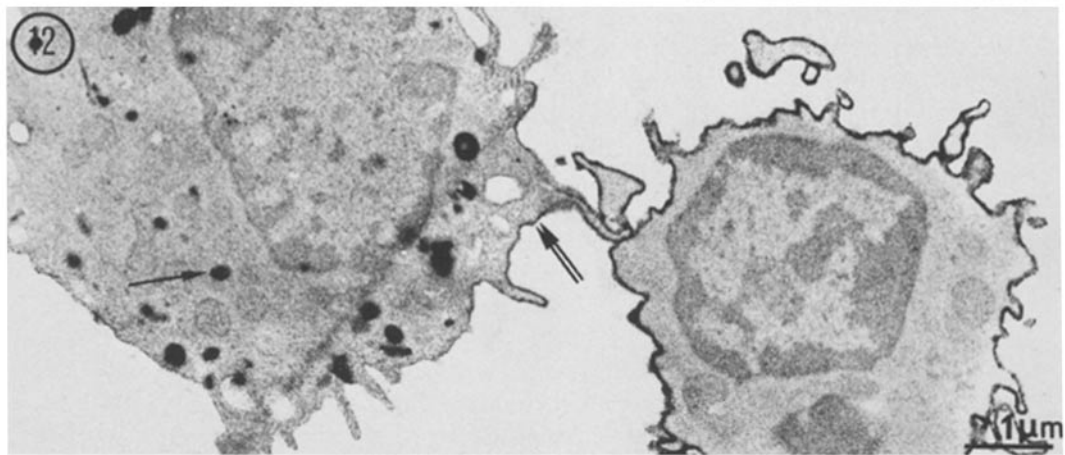
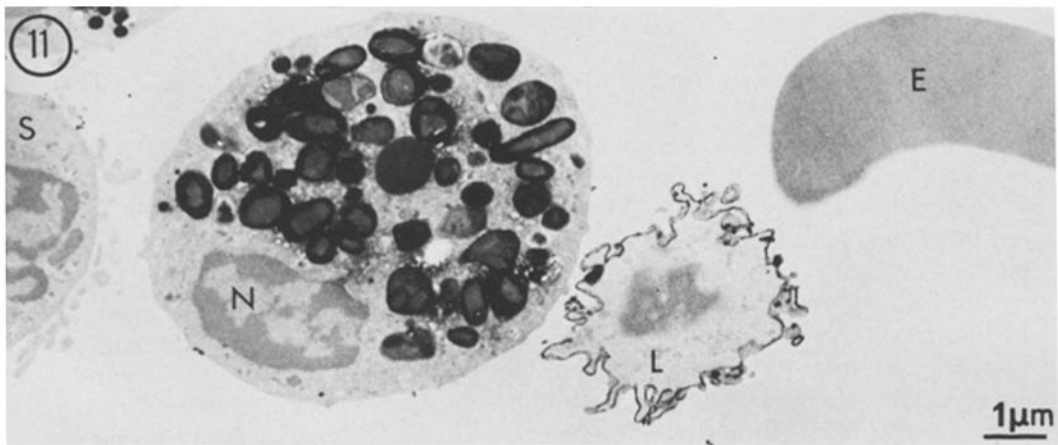
Discussion

These observations establish that the functional heterogeneity of human lymphocytes is associated with morphological surface differences. Separation into T and B lymphocytes is based upon a considerable amount of information and several assays are now available that serve to identify them: among these,

FIG. 8. Normal B lymphocyte with surface IgM. Microvilli are flowing toward one pole of the cell, the remaining membrane being relatively smoother and moderately labeled. N, nucleus. Mi, mitochondria. An intermediate B lymphocyte is seen at upper right, and a neutrophil polymorphonuclear at lower left. The latter exhibits peroxidase-containing granules; its membrane is not labeled. $\times 12,000$.

FIG. 9. This IgM-bearing lymphocyte exhibits a more complete polar concentration of labeled microvilli. The remaining surface is poorly labeled. $\times 12,000$.

FIG. 10. Uropod-bearing lymphocyte exhibiting a "hand-mirror profile". sIg are detected by anti-Ig antibodies on the villous processes of the posterior end of this cell (arrows). A neighboring monocyte is also labeled. $\times 12,000$.



detection of sIg is essential for the recognition of B lymphocytes (17, 18). Here we demonstrate that sIg are present on the villous population of normal blood lymphocytes while smooth lymphocytes have no detectable sIg, thus confirming by immunologic identification previous observations of Polliack and co-workers (7). Preliminary results from this laboratory indicate that normal bone marrow lymphocytes with detectable sIg have also a villous surface (unpublished observations).

Although not quantitative, the distribution of B lymphocytes according to sIg classes is in agreement with previous data resulting from immunofluorescence studies, showing a predominance of IgM-bearing blood lymphocytes (18-20). From such studies of normal and malignant B lymphocytes, sIg have also been shown to be an active product of the cell rather than passively adsorbed immunoglobulins (18).

To date no major morphological distinction of the surface of mammalian lymphocytes has emerged from immunoelectron microscopic studies using the autoradiography technique (4, 5, 21), virus (22), ferritin (23-25), or peroxidase-conjugated antibodies (3, 6, 26). However, it can be noticed that these experiments have been carried out on living cells, thus allowing some surface redistribution and endocytosis of reagents, as a result of membrane activation (26-28). Therefore, early events induced by anti-immunoglobulin antibodies are able to modify the surface morphology of the cell; for instance with peroxidase-conjugated antibodies, internalization of the labeled membrane is a common feature of human B lymphocytes which may then lose their villous character.³ In addition most of the above cited experiments have also been carried out in the cold, a procedure known to lower, but not to suppress (26, footnote 3), activities such as pinocytosis. It has recently been shown that this procedure is also able to reduce significantly the number of lymphocyte microvilli (29).

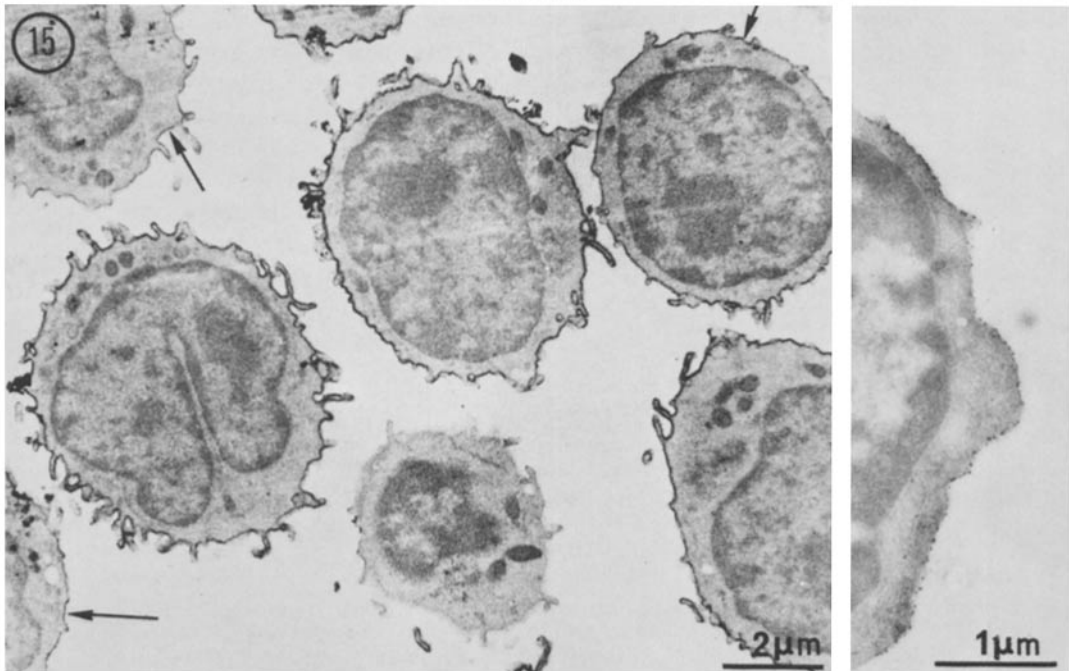
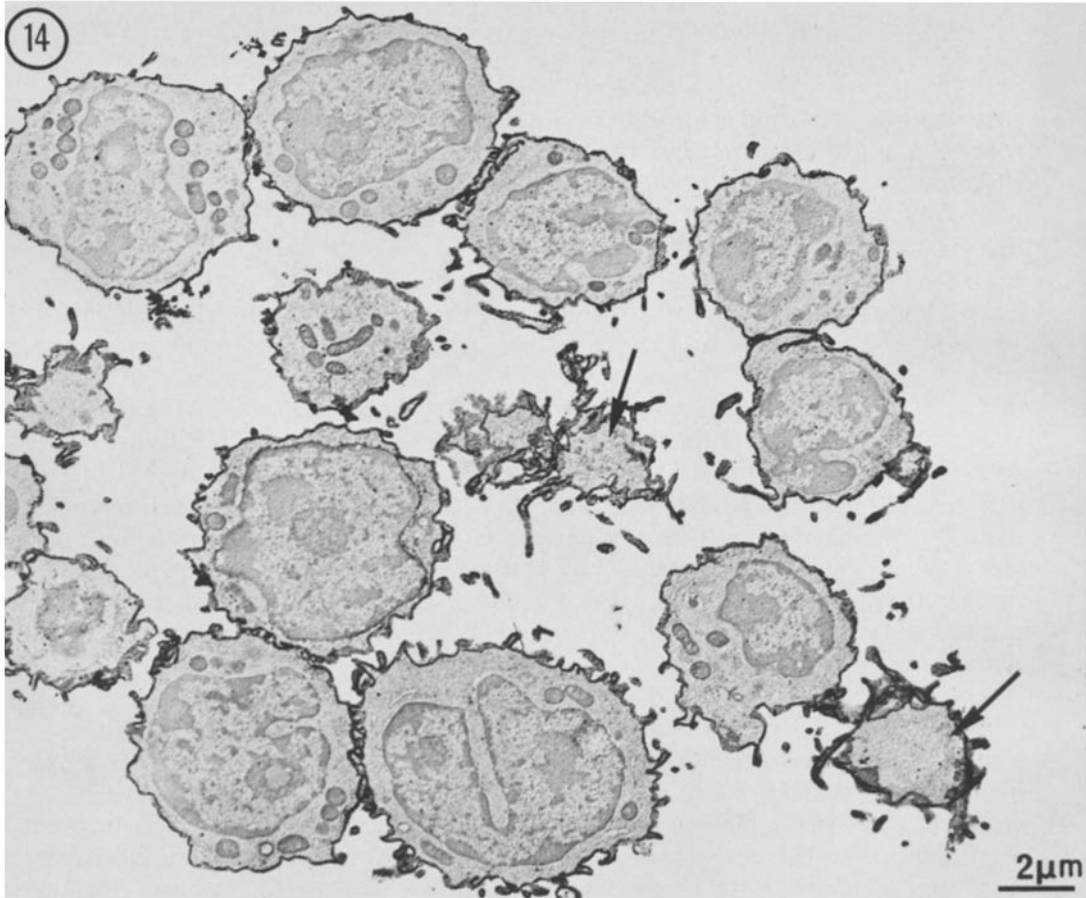
On the contrary, prior fixation allowed the observation of lymphocytes with an unperturbed membrane. It has already been shown that glutaraldehyde fixation does not affect the detection of surface antigens of lymphocytes or erythroid cells, as revealed by immunoelectron microscopy (8, 30). The preservation of cell membrane immunogenicity after glutaraldehyde fixation has been demonstrated independently in other systems (31, 32). Furthermore, isolation of anti-immunoglobulin antibodies can be carried out by fixation and elution from immunoadsorbents in which immunoglobulins have been insolubilized by glutaraldehyde

³Reyes F., J. L. Lejonc, and M. F. Gourdin. Manuscript in preparation.

FIG. 11. Blood buffy coat from Sézary's syndrome exposed to anti-Ig antibodies. Central large cell is an eosinophil polymorphonuclear with endogenous peroxidase located in numerous granules. N, nucleus. The membrane of this granulocyte is not labeled. L, villous B lymphocyte sectionned at one pole. S, Sézary cell with some microvilli and no detectable sIg. E, erythrocyte. $\times 8,000$.

FIG. 12. Villous B lymphocyte with surface IgM. Continuous monocyte contains peroxidase-positive lysosomes (single arrow). Fingerlike projections of this cell are also labeled by anti-IgM antibodies (double arrow). $\times 11,200$.

FIG. 13. Villous B lymphocyte labeled by anti-IgM antibodies. The membrane of the contiguous monocyte is free of labeling. Arrow, peroxidase-containing lysosome. $\times 22,000$.



(11). Therefore, this agent appeared to be a suitable cell fixative in order to preserve sIg. The three specificity controls used in our experiments rule out the possibility of a false labeling resulting from the cross-linking of conjugated reagents by surface residual aldehyde groups.

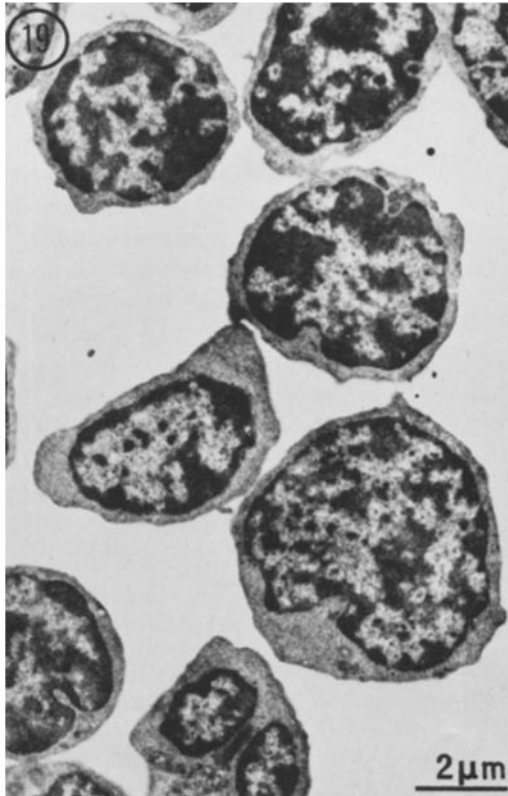
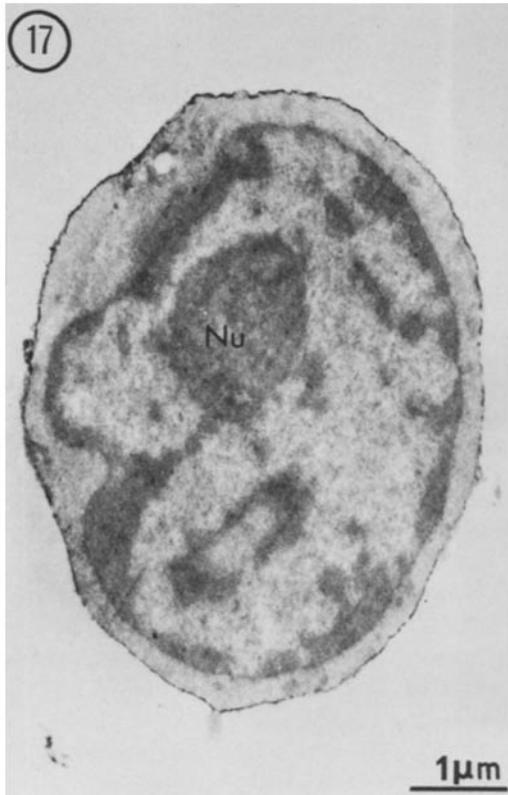
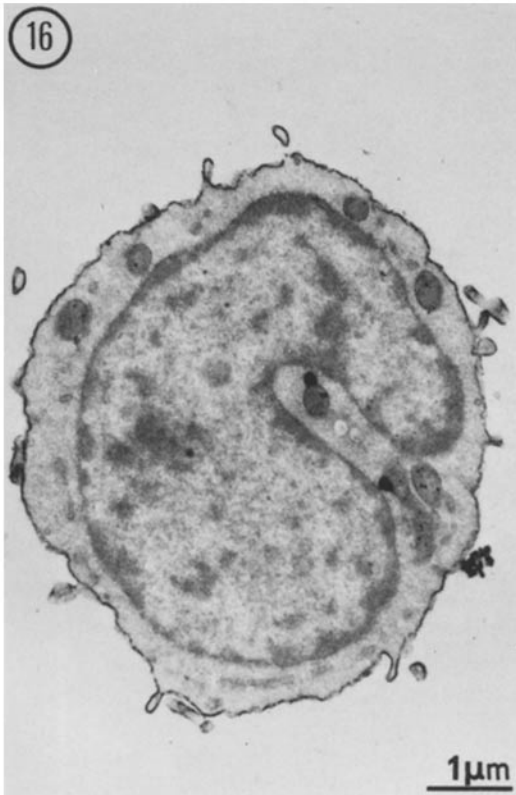
Our observations also indicate that in most cases sIg are distributed all over the surface of normal lymphocytes. This distribution pattern is at variance with previous immunoelectron microscopy observations cited above. In these studies sIg appeared to be confined to separated circumscribed segments of the cell membrane (4, 5, 21-23). In fact this "patchy" distribution observed on living cells has been shown to be artefactual and to result from the clustering of sIg by divalent antibodies (25). This cross-linking of mobile sIg within the fluid membrane is believed to be an essentially passive process followed by active steps, such as polar redistribution (capping) and endocytosis by the cells (for review see 33). It is of interest that when cells are exposed to ferritin-conjugated monovalent antibodies (Fab), sIg are found to be diffusely distributed (25). However, even when uniformly distributed over the cell surface, molecules of ferritin are separated by short gaps of unlabeled membrane (25). This labeling pattern is thus different from the peroxidase staining which gives a continuous deposit outlining the cell surface. Reasons for these differences have been discussed elsewhere (34).

Our observations demonstrate that B lymphocytes have a villous surface. They also provide indirect although strong evidence for smooth blood lymphocytes being T cells. Since Polliack's first report (7) a few papers have appeared in which the surface of T lymphocytes has been investigated after their identification as rosette-forming cells with sheep erythrocytes. Results are conflicting since rosetting T cells have been found smoother than B cells by some investigators (35) but more villous than the remaining lymphocytes by others (36). It is our opinion that examination of the surface of rosetting lymphocytes should be interpreted with caution. The adherence of erythrocytes to surface receptors may alter the lymphocyte membrane status, a process in some way reminiscent of the activation induced by other ligands (37, 38). Very recent observations of rosetting cells by scanning electron microscopy led Polliack and co-workers to the conclusion that rosetting, by altering the cell surface, could account for some "villous" T lymphocytes (39). In addition it remains to be determined to what extent the procedure used to isolate lymphocytes from blood independently of rosetting itself, may alter their normal surface architecture.

Further evidence for a relationship between sIg and microvilli of unaltered cells is provided in our study by the finding of "intermediate" lymphocytes which

FIG. 14. Low magnification micrograph of CLL blood lymphocytes. All cells are heavily labeled by anti-IgM antibodies and have a villous surface. When seen in a tangential plane of section such B lymphocytes have a "hairy" appearance (arrow). $\times 4,900$.

FIG. 15. CLL blood lymphocytes reacted with anti-IgM antibodies. Variations of the staining intensity and of the number of microvilli can be seen, with cells weakly labeled (arrow) and one cell not labeled. $\times 6,400$. Inset, high power view of an artefactually labeled smooth membrane, after exposure of fixed cells to horseradish peroxidase alone. This faint discontinuous staining is easily distinguished from a specific reaction product. $\times 16,000$.



appeared to be poorly villous and weakly labeled by anti-immunoglobulin antibodies. One cannot exclude the possibility that some of them correspond to cells whose microvilli have spontaneously moved to one pole, thus escaping the plane of section. After examination of large numbers of sections however, this feature was rather uncommon suggesting that a very small proportion of resting lymphocytes had undergone such a spontaneous polar movement. It thus appears that a subpopulation of moderately villous lymphocytes exists in human normal blood, as already evidenced by scanning electron microscopy studies (7, 39). The parallel detection of moderate amounts of sIg on these cells allows for their identification as B lymphocytes. This finding also leads one to speculate about the possibility that these cells represent a transitional step between smooth and villous states. Functional transition has independently been proposed as an explanation for the finding of human lymphocytes bearing both B and T markers (40).

The functional implications of B lymphocytes microvilli remain to be determined. At the present time it is tempting to connect this peculiar architecture to other surface properties as revealed by some *in vitro* behavior of these cells. Thus, reports on electrophoretic mobility of human blood lymphocytes indicate that B cells are slower than T cells, i.e. have a different surface charge (41). The finding of B-lymphocyte adherence to glass beads or nylon wool must also be considered. This property is now widely recognized for human (42, 43) and animal (44-48) B lymphocytes. Microvilli may well be the morphological correlate of this surface property, assuming that adherence is in relation to the presence of sufficient contact areas of the cell surface. Interestingly such an adherence of B lymphocytes can be inhibited by exposure of cells to sodium azide and cold (43), procedures known to affect energy-requiring membrane activities (33) and to lower the number of surface microvilli (29).

The concentration of microvilli at one pole of B cells may be related to uropod formation, a locomotion feature of lymphocytes (49, 50). It must be stressed that in our experiments such a polar concentration arose spontaneously before exposure of the cells to reagents. Uropod formation has been previously ascribed to T lymphocytes (26) but recent evidence indicate that B lymphocytes are also induced to move in a similar pattern, for instance after exposure to anti-immunoglobulin antibodies (51). Relations between this latter phenomenon and capping of sIg are not completely clarified (51). However, the finding of labeled microvilli at the posterior end of the "hand mirror" B lymphocyte (Fig. 10) suggests that this concentration of microvilli may be an essential step in both phenomenon.

FIG. 16, 17. Blastic cells from CLL blood, with detectable surface IgM. $\times 12,000$. A thin layer of chromatin is margined at the nuclear membrane. A large nucleolus is seen (Nu). Surface is smooth (Fig. 17) or exhibits rare short microvilli (Fig. 16).

FIG. 18. Blood Sézary cell with a typical convoluted nucleus. Cytoplasm contains small lysosomes, lucent pinocytotic vesicles (single arrow) and multivesicular bodies (double arrow). Surface microvilli are apparent with no staining after exposure to anti-Ig antibodies. Section counterstained by lead citrate. $\times 9,700$.

FIG. 19. Normal thymocytes exposed to anti-Ig antibodies. There is no labeling of their regular smooth membrane. $\times 6,000$.

The present study of blood and bone marrow CLL lymphocytes gives results which are in agreement with previous immunofluorescence data indicating that in most cases CLL is identifiable as a B-cell malignancy (52, 53) with a monoclonal distribution of sIg (53). However, some findings deserve additional comments.

Although variations were noted between patients, a general weak labeling was found in several cases of this study. This observation is in agreement with previous morphological data suggesting that the amount of sIg is lower in malignant than in normal B lymphocytes (52, 53). Recent quantitative studies have led to a similar conclusion (54). In addition we also found individual cell variations of the anti-immunoglobulin-binding capacity in every sample. Thus, it can be noted that the cellular proliferation does not preclude some degree of heterogeneity with regard to the B-cell maturation stage as judged by the amount of detected sIg, even if in CLL B lymphocytes are basically affected by a block in their maturation process (18).

As mentioned above a direct relationship was found in CLL between the labeling intensity and the number of microvilli of most B lymphocytes, as in normal blood. However, a definite proportion of blastic cells was found in two CLL samples, which had a smooth surface and detectable sIg. The presence of small amounts of blastic cells among the lymphocytic infiltration is a possible feature of CLL. In other studies such lymphoblasts have also been shown to carry the same sIg as remaining B lymphocytes and thus to belong to the same proliferative process (53). However, the significance of this cell type in terms of B-cell line maturation remains unclear. There is now a need to study the surface architecture with parallel immunologic identification through the lymphocyte maturation pathway.

In this study abnormal blood lymphocytes termed as "Sézary cells" had no detectable sIg. Sézary's syndrome is characterized by a generalized erythrodermia, lymphadenopathies, and an infiltration of skin by these abnormal cells which also circulate in blood. By several immunological assays it has recently been shown that Sézary cells have membrane properties of T lymphocytes (55, 56). However, the lack of sIg reported here also concerned the segments of villous membrane exhibited by these lymphocytes. This finding makes another exception to the general relationship found in this study.

As already mentioned the various blood and marrow samples contained a few contaminating granulocytes and monocytes displaying some surface labeling. Receptors for immunoglobulins have been detected on the surface of such mammalian leukocytes in experiments involving living cells (57-61). These cells are therefore able to bind circulating immunoglobulins *in vivo* and could as well bind some rabbit antibodies *in vitro*, thus exhibiting nonspecific labeling. In this study, the latter possibility is ruled out since control experiments with normal conjugated rabbit γ -globulins gave constant negative results. An alternative hypothesis would be a "false" labeling of contiguous cells by flowing of the enzymatic reaction product from positive lymphocytes (6). This seems improbable here since labeled monocytes were found which were not contiguous to B lymphocytes and conversely cells in close contact were devoid of surface staining (Fig. 13). Finally, labeling of contaminating leukocytes was found when

cell suspensions had been exposed to conjugated anti-Ig, anti-IgM or anti-IgG antibodies suggesting that these reagents were detecting immunoglobulins adsorbed on these leukocytes before fixation. However, their presence as a result of in vivo adsorption is questionable since such cytophilic immunoglobulins when bound as free molecules are expected to elute easily from the cell membrane during washing steps (57). An alternative possibility would be an adsorption of sIg after their release by lymphocytes in vitro just before the fixation step.

Summary

Surface immunoglobulins (sIg) were detected on human lymphocytes by immunoelectron microscopy with peroxidase-conjugated antibodies. Blood, marrow, and thymus cells from normal individuals and patients with lymphoproliferative disorders were examined. Samples were fixed before exposure to specific reagents. Normal lymphocytes with detectable sIg, i.e. B lymphocytes, were characterized by a villous surface; nonlabeled blood lymphocytes and thymocytes were smooth cells. Intermediate cells were also found which in sections appeared moderately villous and labeled, thus identified as B lymphocytes. Further evidence for a relationship between villous surface and sIg was given by the finding of a few lymphocytes with polar concentration of labeled microvilli.

In chronic lymphocytic leukemia patients, most cells exhibited a villous surface with parallel variations of the number of microvilli and of anti-immunoglobulin-binding capacity. However, some labeled smooth blastic cells were also observed. On the other hand, abnormal lymphocytes from Sézary's syndrome which could exhibit segments of villous membrane had no detectable sIg. This study confirms that in most cases human B lymphocytes have a characteristic surface appearance and that the detection of sIg in normal lymphocytes correlates with the presence of microvilli.

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