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Human lymphocyte surface immunoglobulin capping. Normal characteristics and anomalous behavior of chronic lymphocytic leukemic lymphocytes.

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Research Article

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Human Lymphocyte Surface Immunoglobulin Capping

NORMAL CHARACTERISTICS AND ANOMALOUS BEHAVIOR OF CHRONIC LYMPHOCYTIC LEUKEMIC LYMPHOCYTES

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ABSTRACT The phenomenon of redistribution of surface membrane immunoglobulin (Ig) components (capping) has been well described in mouse lymphoid cells. The characteristics of this process in human lymphocytes are less clear. This study characterizes the phenomenon of surface membrane Ig redistribution of normal and chronic lymphocytic leukemia (CLL) lymphocytes with the use of fluoroscein-labeled anti-Ig sera. Normal lymphocytes underwent rapid cap formation after incubation with anti-Ig serum in the cold and subsequent rewarming. The morphology was characteristic with aggregation over the pole of the cell opposite the nucleus and over the uropod when present. The process was energy dependent but independent of protein synthesis, and could be inhibited by vincristine, vinblastine, and colchicine but not by cytochalasin B. CLL cells, on the other hand, though showing fluorescent complex aggregation on the surface, rarely demonstrated unidirectional movement of these aggregates to form a cap. Cap formation in these cells could not be stimulated by supplementing the energy source or protein concentration of the medium nor by adding glutamic acid which could partially reverse the vincristine and vinblastine inhibition of normal capping. The failure of agents which inhibit motility to inhibit capping of the normal lymphocytes suggests that active locomotion is not a direct prerequisite for capping. The results also suggest the involvement of microtubules in normal capping and the possibility that abnormal membrane structure or microtubular function could explain the failure of CLL cells to behave normally in this regard. The role of this cellular defect in the immune deficiencies exhibited by many patients with CLL, however, is not established.

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INTRODUCTION

It is now clear that the circulating peripheral blood lymphocytes are a heterogenous group of cells having different biological properties and functions. One population contains easily detectable immunoglobulin (Ig)[1] as a component of the surface membrane and has been defined as the "B cell" or bone marrow-derived population mainly through studies of mouse lymphocytes (1, 2). Studies of human cells utilizing other surface markers as indicators and patients with immunodeficiency diseases (3) confirm this population among normal human peripheral blood lymphocytes and have demonstrated that they generally constitute somewhat less than 25% of normal circulating lymphocytes (4, 2). Further investigation of the Ig's in the surface membrane has resulted in the description of the "capping phenomenon" (5–7). This phenomenon involves the temperature-dependent redistribution of these surface moieties induced by an anti-Ig, Ig reaction at the surface and generally monitored by fluorescent antibody or radioactively labeled antibody techniques. The phenomenon has been well characterized in mouse lymphocytes and shown to be both energy and temperature dependent (5, 8–10). These observations have been consistent with the formulation of the "fluid mosaic" concept of surface membrane structure which may apply to many normal cell types and surface markers (11, 12). This hypothesis suggests that protein moieties of the cell membrane float freely within the lipid components of the membrane and are free to move randomly in lateral motion about the cell surface. Capping has also been observed in a variety of cell types and may be a general property of animal cells (13). It has been suggested that the redistribu-

[1] *Abbreviations used in this paper:* B, bursal equivalent or bone marrow-derived lymphocyte; CLL, chronic lymphocytic leukemia; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; Ig, immunoglobulin; M199, tissue culture medium 199; M199-G, tissue culture medium 199 +0.1% gelatin; T, thymus dependent.

tion of such receptors observed after antibody-antigen reactions at the cell surface of lymphocytes might play a role in the initiation of the antibody response (14). However, studies of human lymphocytes with respect to surface Ig redistribution have been limited and contradictory (15–17).

Recent studies in this and other laboratories have indicated that in patients with chronic lymphocytic leukemia (CLL) the peripheral blood lymphocytes are comprised predominantly of cells containing surface membrane Ig (18–21). This observation has prompted some to consider the CLL lymphocyte a B cell derivative (22). However, such patients generally have decreased circulating Ig and antibody responses despite the increased number of surface membrane Ig-containing cells, indicating a functional B-cell immunodeficiency.

This study was undertaken to further define the surface Ig characteristics of human lymphocytes by studying the characteristics of the capping phenomenon in normal lymphocytes and CLL lymphocytes. The results demonstrate that normal human lymphocytes do undergo capping, i.e. redistribution of surface membrane Ig after the reaction with anti-Ig antibodies, whereas CLL lymphocytes are markedly deficient in this regard. Further characterization of this phenomenon in normal human lymphocytes employing various inhibitors suggests the involvement of certain cellular organelles, and a possible mechanism for the CLL cell abnormality.

METHODS

Lymphocyte preparation. 20 cc of blood were removed from normal volunteers and untreated patients with CLL into a plastic syringe containing approximately 100 units of Heparin (Upjohn Co., Kalamazoo, Mich.) and the red cells sedimented with the addition of 20% vol/vol of plasmagel (HTI Corp., Buffalo, N. Y.). A white blood cell-rich supernate was removed, incubated with 1 g of carbonyl iron powder (GAF Corp., Atlanta, Ga.)/10 ml of blood in a 600-ml plastic beaker and agitated for 20 min in a 37°C water bath. The lymphocyte-rich supernate was poured off, while the iron particles were retained with a magnet, and centrifuged for 30 min at 800 *g* at room temperature through a Ficoll-Hypaque gradient (9% Ficoll [Pharmacia Fine Chemicals, Piscataway, N. J.], 50% Hypaque [Winthrop Laboratories, New York], 3:1) as modified from Boyum (23). Cells from the plasma-Ficoll interface were washed once with phosphate-buffered saline, pH 7.2 and four times with tissue culture medium 199 + 0.1% gelatin (M199-G) (Grand Island Biological Co., Grand Island, N. Y.). All separations and subsequent incubations were performed in plastic tubes and with plastic pipettes (Falcon Plastics, Oxnard, Calif.). In the case of some patients with CLL with extremely high lymphocyte percentages, the iron incubation to remove phagocytic cells was unnecessary. The cell preparations thus obtained consisted of more than 99% lymphocytes and were always greater than 95% viable by trypan blue exclusion. Lymphocyte recovery was approximately 70%.

Fluorescent antibody characteristics. Fluorescein-labeled antisera, with specificity for human IgG, IgM, IgA, kappa, and lambda light chains contained in a single polyvalent antiserum, were obtained from Meloy Laboratories Inc. (Springfield, Va.) and Hyland Div., Travenol Laboratories, Inc. (Costa Mesa, Calif.). The specificity of the antiserum was assured by determining that the polyvalent antisera reacted only with human Ig's by immunoelectrophoresis. In addition the fluorescent labeling of cells could be specifically inhibited by preincubation of the antisera with the appropriate Ig mixture. The fluorescent studies to be described generally used a total of 20–100 μg of antibody in the incubation mixture. The fluorescein: protein ratios of the antisera were generally 3:4.

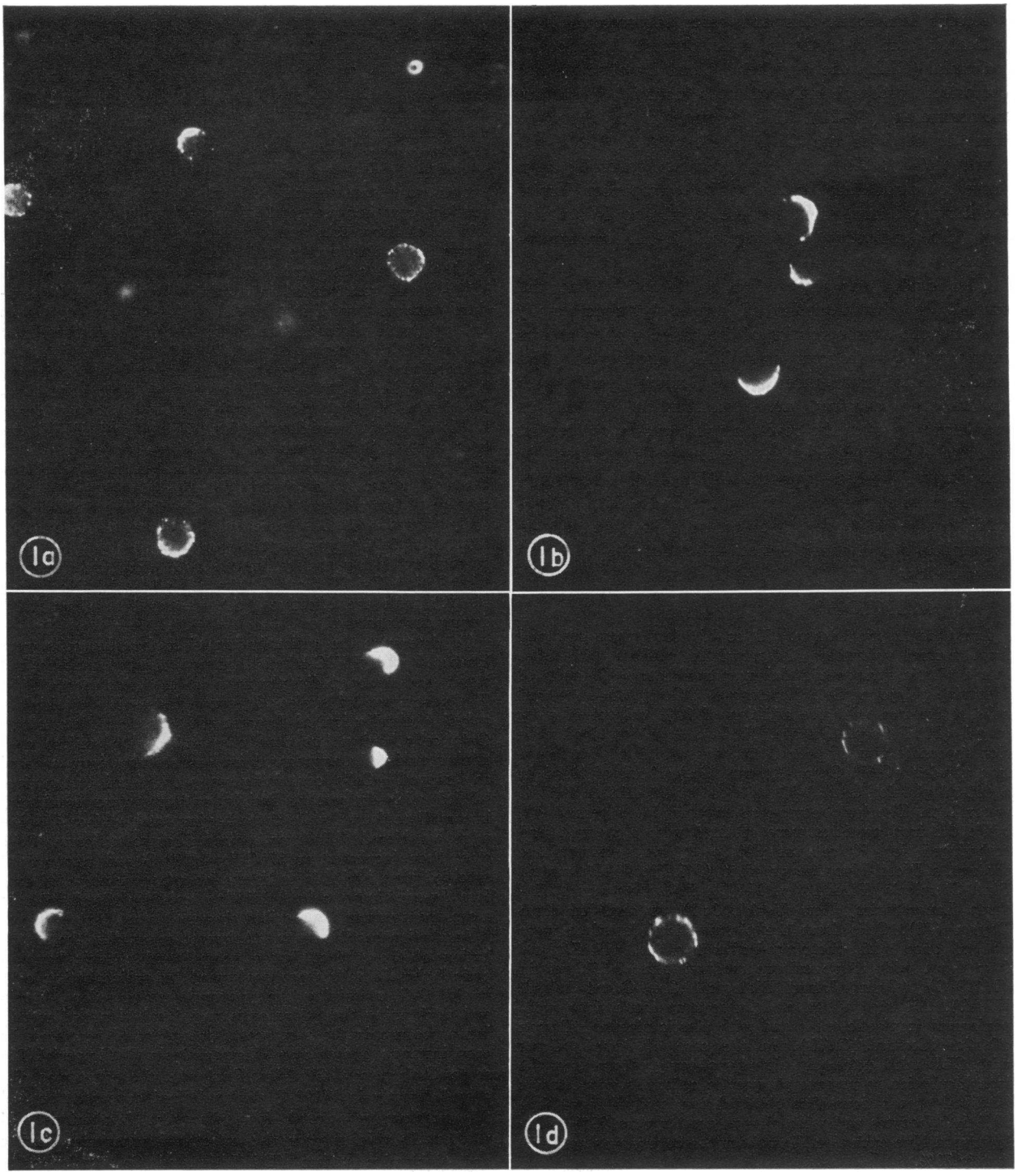
Fluorescence labeling of cells. All incubations, unless otherwise noted, were performed using M199-G without an exogenous serum source. 0.05 ml of the lymphocyte suspension, containing a total of 2×10^6 cells, was added to 0.05 ml of fluorescein isothiocyanate (FITC)-labeled anti-Ig serum in a plastic tube and incubated for 45 min in an ice bath. The cells were then washed three times in cold M199-G and resuspended in a minimal volume. All operations were performed at 4°C and the cells were kept in an ice bath until observed for fluorescence. Fluorescence was determined by placing a drop of labeled cell suspension on a microscope slide, covering it with a coverslip, and viewing it with a Leitz Ortholux II microscope (E. Leitz, Inc., Rockleigh, N. J.) equipped with a HB200 mercury lamp, a Ploem vertical epi-illumination system, and FITC exciter filters. Simultaneously, a substage tungsten-halogen light was used to count the total number of lymphocytes. Thus, in order to determine the total number and percentage of lymphocytes fluorescing, the cells were simultaneously viewed with the visible and fluorescent light source, a total of 200–300 lymphocytes were counted, and the percentage exhibiting surface fluorescence noted. Pictures were taken using Tri X film (Eastman Kodak Co., Rochester, N. Y.) with the effective ASA increased to about 1,200 during development.

To determine the redistribution of surface Ig, cells were incubated with FITC-labeled antisera, washed as described above, and removed directly from ice to a microscope slide. The percentage of surface Ig-containing cells exhibiting a unipolar distribution of fluorescence was then determined. Only cells having less than one-third of the surface containing uniformly aggregated immunoglobulin were counted as "capped." Cells containing up to 75% of the surface labeled with fluorescent anti-Ig antisera were considered intermediate forms while those having total surface labeling were considered diffuse or spotted fluorescent forms. Cells were then incubated at 37°C in M199-G and removed at specified time intervals to determine the percentage of surface Ig-containing cells exhibiting unipolar distribution in a cap arrangement as described above.

Incubation modifications. Attempts to modify the distribution of the surface Ig on normal and chronic lymphocytic leukemia cells were performed by preincubating cells in M199-G containing the appropriate agent for 10 min and performing the entire antiserum incubation and warming procedure in the presence of the agent to be tested. In each instance, except that of cytochalasin B (Imperial Research Laboratory, Cheshire, England), the agent was dissolved directly in M199-G at the desired concentration and this medium was used for subsequent incubations and washes. In this manner the effects of vincristine sulfate and vinblastine sulfate (Eli Lilly and Co., Indianapolis,

Ind.), colchicine (Sigma Chemical Co., St. Louis, Mo.), puromycin (Sigma Chemical Co.), and sodium azide (Fisher Scientific Co., Springfield, N. J.), were tested.

Cytochalasin B was first dissolved in 23% ethanol (EtOH) at a concentration of 190 μg/ml. Dilutions of 1:4, 1:5, 1:10, and 1:20 were made with M199-G to give final cytochalasin B concentrations of 48 μg/ml, 38 μg/ml, 19 μg/ml, and 9.5 μg/ml in the resultant EtOH dilution. The EtOH concentration was a maximum of 5.7% EtOH/M199-G. Incubations were performed as described above.



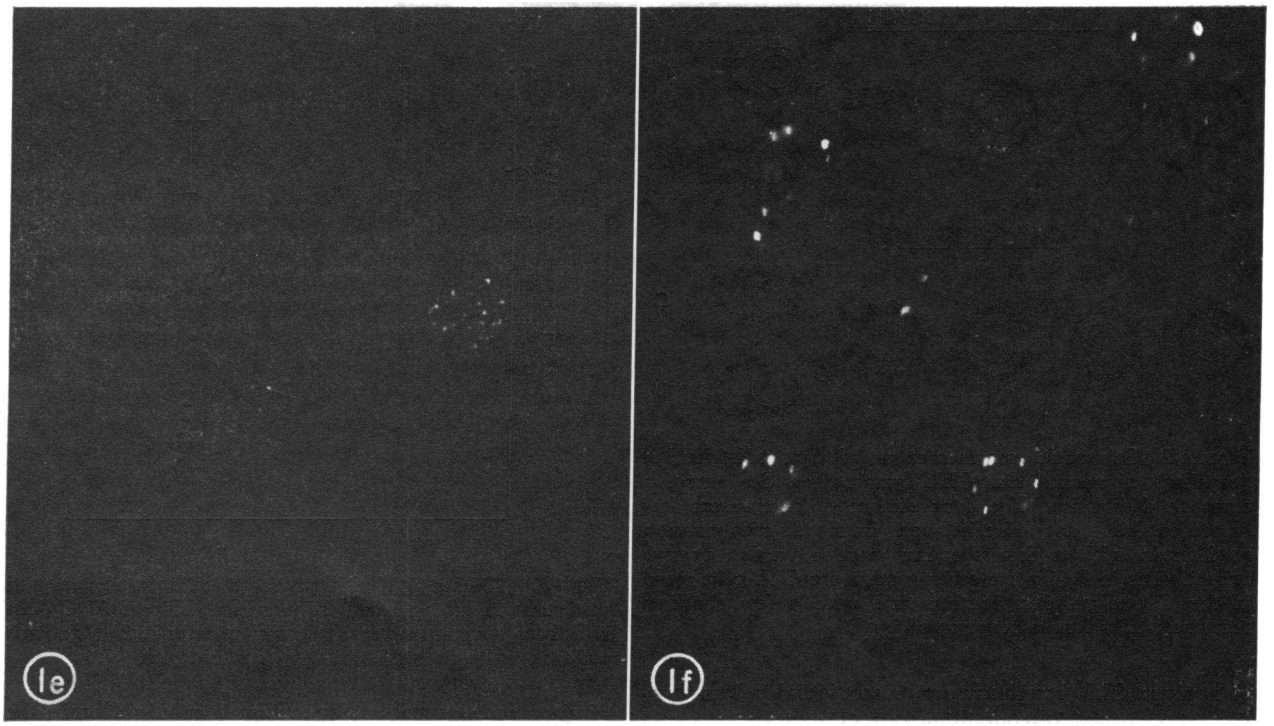


FIGURE 1 Fluorescence microscopy of normal and CLL cells after incubation with FITC-anti-Ig. All pictures are taken at original magnification of × 540 with a tube reduction factor of 0.32 and magnification in photographic processing approximately × 4.5. (*a*) Normal lymphocytes viewed immediately after incubation at 0°C. Most fluorescent cells exhibit fluorescence distributed about the surface. (*b*) Normal lymphocytes after 15 min incubation at 37°C showing the typical capped appearance. (*c*) Normal lymphocytes after 30 min incubation at 37°C. This picture is taken with low-level visible light as well as transmitted fluorescent light in order to show the typical capped morphology with distinct surface polarity. Some of the cells, especially lower left and upper right, exhibit early uropod formation. (*d*) CLL cells at 0°C. The fluorescence is distributed around the surface with some small areas of aggregate formation. (*e*) CLL cells after 2 min incubation at 37°C. There are multiple small areas of aggregation with clearing of the surface between. This degree of low-level fluorescence was frequently seen on CLL cells. (*f*) CLL cells after 30 min incubation at 37°C. The fluorescence continues to be distributed about the surface but the aggregates are larger and there are larger clear areas between. (See text for further description.)

Control samples were treated with EtOH in M199-G at concentrations corresponding to those in the cytochalasin B dilutions (maximum 5.7%).

Supplemented media were prepared by adding sufficient material to M199 to create the following final concentrations: fetal calf serum (FCS) (Grand Island Biological Co.) 15%; human albumin (E. R. Squibb and Sons, New Brunswick, N. J.) 15%; bovine serum albumin (Sigma Chemical Co.) 15%; sodium *l*-glutamate (Sigma Chemical Co.) 10^{-2}–10^{-4} M; and glucose 3%.

Statistical methods. All comparisons were made by Student's *t* test for unpaired variables.

RESULTS

A total of 23 patients with CLL and 20 normal controls were studied. The latter included a spectrum of ages although they were not specifically age matched. As previously reported (18) normal individuals always had less than 30% surface Ig-containing lymphocytes. On the other hand, cells from CLL patients demonstrated a wider spectrum from a small number of patients containing under 10% labeled cells to others with 90% surface Ig-containing cells; the mean was 75% (18). Studies of the quantitative aspects of surface Ig, complement, and sheep red blood cell receptors in CLL will be reported elsewhere.[2]

Membrane immunoglobulin distribution. When viewed immediately at 0°C, 95% of the fluorescent lymphocytes among both the CLL and normal individuals had a diffuse or finely spotted ring surface pattern (Fig. 1 *a* and *d*). Often the intensity of fluorescence

[2] Cohen, H. J. Lymphocyte surface receptors in CLL. Manuscript in preparation.

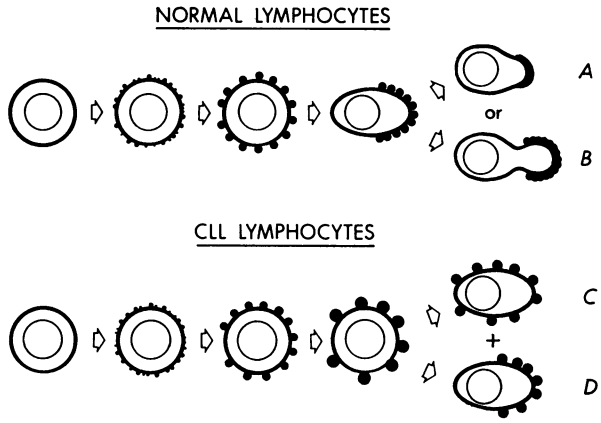


FIGURE 2 Schematic representation of Ig redistribution (capping) of normal and CLL lymphocytes. See text for complete description. The dark surface dots represent fluorescence. Most normal cells were in the A configuration at 30 min with a few showing more extensive uropod formation as in B. On the other hand, most CLL cells were in the C configuration at that time with a few showing some redistribution as represented by D.

of a given CLL lymphocyte was considerably less than that of a normal Ig-containing cell (Fig. 1 *e*).

Upon warming the incubation mixture to 37°C a characteristic sequence of changes was observed in the progression of cells from the ring to the cap form. This differed considerably between normal and CLL lymphocytes. Initially most fluorescent lymphocytes of both groups exhibited diffuse or finely spotted ring fluorescence (Fig. 1 *a* and *j*). Within 2 min many of the cells (both normal and CLL) showed aggregation of the small fluorescent spots into spots that became somewhat larger and separated by a greater amount

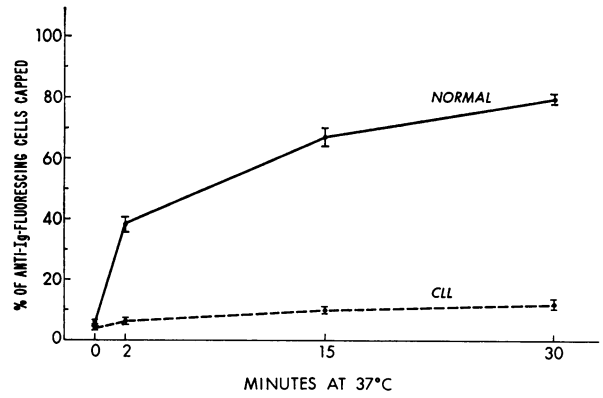


FIGURE 3 Time-course of surface Ig redistribution of normal and CLL cells. After incubation with FITC-anti-Ig at 0°C, cells were placed at 37°C and viewed at time intervals as described in Methods. The points represent the mean values ± SEM for all CLL and normal patients studied.

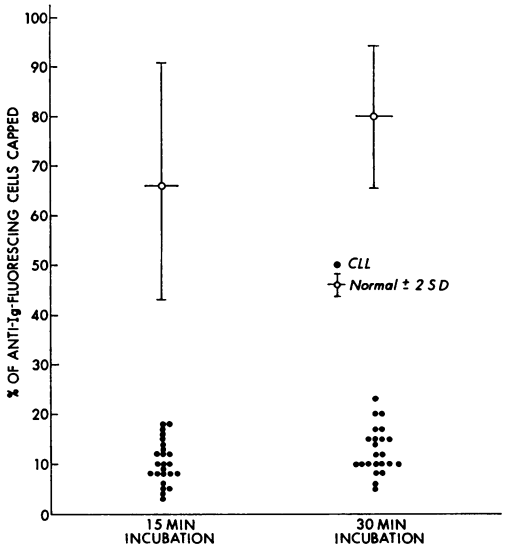


FIGURE 4 Percentage of fluorescent cells in the capped configuration after 15 and 30 min incubation at 37°C. The bars represent the normal population mean ±2 SD. Each patient's value is represented by a single dot.

of clear area on the surface (Fig. 1 *e*). At 15 min the majority of fluorescent normal cells demonstrated clearing of two-thirds of the cell surface with fluorescence remaining at one pole of the cell often tightly aggregated at this pole (Fig. 1*b*). At this time 80% of the CLL cells remained in a spotted fluorescent distribution but the aggregates appeared somewhat larger than before and fewer in number. At 30 min the CLL pattern remained the same but more than 75% of the normal cells were in a capped configuration with many of them occupying even less surface area than before (Fig. 1 *c* and *f*). When normal cells were in the capped configuration the fluorescence formed a homogenous border of the part of the cell occupied (*Fig.* 1 *b* and *c*).

Normal cells were frequently observed to have an elongated bulging process at one end of the cell with a constriction delineating it from the rest of the cell. This elongation, characteristically occurring in lymphocytes at the posterior pole of a moving cell, opposite from the nucleus as in the cells described herein, is called a uropod (24). Capping was often seen in such cells and when it occurred was always over the uropod end of the cell. Such polarity was observed both in cells floating freely in the medium and those adhering to the surface of the slide (Fig. 1 *c*). This configuration of the cells was rarely observed among CLL lymphocytes and when caps did appear they seemed to be at a random distribution relative to the nucleus. Moreover, the caps appearing on the surface of CLL cells rarely exhibited the uniform coalescence of fluorescent

material demonstrated in normals and seemed to be closer aggregations of the larger fluorescent spots. These various configurations are shown schematically in Fig. 2.

Determination of the proportion of cells in the capped configuration demonstrated the difference between CLL and normal cells within 2 min (Fig. 3). At this time an average of 38% of normal surface Ig-containing lymphocytes were in the capped configuration (one-third or less of the surface area demonstrating fluorescence in a homogenous area) while only 6% of the CLL cells appeared in this way. The difference was further accentuated at 15 and 30 min when the normals reached 67 and 80%, respectively, while the CLL lymphocytes reached a maximum of 12%. The individual patients with CLL are depicted in Fig. 4. It can be seen that at 15 and 30 min incubation all of the CLL patients' capping responses were considerably more than 2 SD below the normal means. The differences shown in Figs. 2 and 3 are all significant at a P value of less than 0.0001. Moreover, no CLL patient fell within the observed normal range at 15 and 30 min.

After 30 min of incubation at 37°C much of the fluorescence began to disappear from the surface of both CLL and normal cells with most cells showing little fluorescence after 1 h of incubation. The normal cells appeared to lose the fluorescence somewhat more rapidly, beginning at the 15–30-min interval, but the process occurred in CLL cells as well, despite the lack of capping. Both endocytosis and sloughing of the surface aggregates appeared to participate and though clear evidence of internalization of the fluorescence could be seen, the predominant mechanism of loss was not determined.

To further assess the characteristics of normal cells with the hope of gaining insight to the possible mechanism of the impaired capping phenomenon in CLL, a series of potential inhibitors was studied. The results are shown in Table I. As reported by others in the mouse lymphocyte system (5), sodium azide and potassium cyanide (KCN), inhibitors of energy metabolism, produced a striking reduction in capping while puromycin, an inhibitor of protein synthesis did not. In addition, it was found that vincristine, vinblastine, and colchicine all markedly reduced the percent of capping. On the other hand, cytochalasin B at concentrations of 9.5, 19, and 38 μg/ml failed to inhibit the capping of normal lymphocytes, while the latter concentration of this agent was able to inhibit lymphocyte locomotion thus insuring delivery of an effective dose.[3] At the next concentration, 48 μg/ml, the cytochalasin B proved to be quite toxic and caused greater than 60% cell death by trypan blue exclusion thus making any evaluation of capping unreliable. EtOH in M199-G at concentrations up to 5.7%, the highest present in the cytochalasin B preparations, had no effect and was nontoxic. In all other experiments cell viability, as determined by trypan blue exclusion, was always greater than 90% at the completion of the experiment. In all instances involving inhibition of capping, aggregation of the fluorescent complexes was observed but the fluorescence remained in small aggregates distributed about the surface more or less uniformly. Since the effect of vinblastine on mitotic activity has been reported to be counteracted to some extent by glutamate (25), incubations were performed with vincristine and vinblastine in the presence of sodium glutamate. At concentrations of 10^{-3} and 10^{-4} M no effect was seen. However, as shown in Table II, at a concentration of 10^{-2} M, glutamate could counteract the inhibitory effects of vincristine and vinblastine on the capping phenomenon. This concentration is considerably greater than that present in M199-G (less than 10^{-3} M) and is compatible with the marked concentration excess required for the in vivo effect previously reported (25). On the other hand, glutamate had no effect upon the capping inhibition caused by KCN and sodium azide.

In order to determine whether the lack of capping by CLL cells could be the result of a nutritional re-

TABLE I

Inhibition of Normal Lymphocyte Surface Ig Capping

Addition	% Capped cells	% Inhibition
None (control)	70	—
Sodium azide (10^{-2} M)	5	93
KCN (10^{-3} M)	7	90
Puromycin (50 μg/ml)	65	8
Vincristine (10^{-5} M)	9	87
Vinblastine (10^{-5} M)	8	86
Colchicine (10^{-5} M)	8	86
Cytochalasin B (38 μg/ml)*	69	2

In this experiment 2×10^6 normal lymphocytes in each tube were washed into M199-G containing the indicated concentration of the agent to be tested and incubated for 15 min at 0°C. These cells were then incubated with FITC-anti-Ig for 45 min at 0°C, in plastic tubes as described in Methods, then washed using cold inhibitor medium of the same composition. The tubes were then incubated for 15 min at 37°C and the cells observed immediately as described. The results are expressed as the percentage of fluorescing cells in the capped configuration.

* Highest nontoxic cytochalasin B concentration (see text).

[3] Jarvis, S. C., H. J. Cohen, and R. Snyderman. Motility characteristics of normal human lymphocytes and chronic lymphocytic leukemia cells. Manuscript in preparation.

TABLE II

Effect of l-Glutamate on Inhibited Normal Capping

Addition	% Capped cells	
	Without *l*-glutamate	With *l*-glutamate
None	69	69
Vincristine (10^{-5} M)	8	44
Vinblastine (10^{-5} M)	9	53
Sodium Azide (10^{-2} M)	7	9
KCN (10^{-3} M)	8	7

These experiments were performed as described in legend to Table I except that a series of media were prepared simultaneously containing 10^{-2} M sodium *l*-glutamate in addition to the indicated concentration of inhibitor.

quirement not provided by our media, a number of supplements were employed and the results are shown in Table III. As can be seen, neither the addition of serum proteins or extra energy source had any stimulatory effect, nor did they have any inhibitory effect upon capping of normal cells. Moreover, sodium glutamate produced no augmentation in the percentage of CLL cells capping (Table III).

DISCUSSION

The capping phenomenon, or redistribution of surface membrane antigens, has been well described using mouse lymphocytes (5–7). It is felt, however, to be a more universal property of cellular membranes since it has been demonstrated in fibroblasts, polymorphonuclear leukocytes, and tumor cells (6, 10, 26). The redistribution in mouse lymphocytes as previously described (5–7), may be mediated by divalent antibody or antigen binding to the appropriate receptor (5, 16, 27) and appears to occur in two stages. The first stage, which is temperature and energy independent, involves the rapid formation of small aggregates uniformly distributed over the surface of the cell. The next phase, which requires energy and an elevation of temperature to 20°–37°C, involves the directional movement of the cross-linked and aggregated receptors to one pole of the cell.

TABLE III

Effect of Media Supplements on Capping

Addition	% Capped cells	
	Normal	CLL
None	68	10
Sodium *l*-glutamate (10^{-2} M)	67	9
FCS (15%)	66	14
Human albumin (15%)	69	15
Bovine serum albumin	70	15
Glucose (3%)	65	11

Individual supplements were adjusted to concentrations indicated in M199-G as described in Methods. Cells (2×10^6) in each tube were washed with the indicated medium at 0°C then incubated with FITC-anti-Ig serum in the same medium at 0°C for 45 min as in Methods. The tubes were then incubated at 37°C for 15 min and the cells observed as described in Methods. The values are expressed as the percent of fluorescing cells in the capped configuration at 15 min.

Studies of normal human lymphocyte capping have been limited and contradictory. Preud'Homme, Neauport-Sautes, Piat, Silvestre, and Kourilsky (16) reported that both IgM and HL-A surface components would independently redistribute after reacting with a specific fluoroscein-labeled antiserum to form typical caps. Knapp, Bolhuis, Radl, and Hymans (17) also qualitatively demonstrated independent redistribution of IgD and IgM on human lymphocyte surfaces. On the other hand, Ault, Karnovsky, and Unanue (15) studying the lymphocytes from normal subjects failed to demonstrate the capping phenomenon and suggested that human and mouse lymphocytes differ in this regard. The present study, however, clearly demonstrates that normal human peripheral blood lymphocytes exhibit the capping phenomenon, as previously described for mouse cells, and that it follows similar kinetics. Although the relative protein concentration of the medium has been reported to influence Ig redistribution (5, 9), the divergent findings cannot be explained in this way since the addition of FCS or other protein sources to M199-G was without effect. On the other hand, differences in the antiserum: surface Ig ratio have also been reported to influence capping (5, 9) and the variant results may relate to the specific properties or concentrations of the FITC antisera employed in the study, e.g. antibodies to more Ig determinants, or other less obvious culture conditions. However, our demonstration of the polarity of many of the normal cells with cap formation over the uropod appears to clearly establish the existence of this phenomenon in normal human lymphocytes.

Our inhibitor studies confirm that the second phase of the capping process of human lymphocytes, as previously demonstrated in the mouse lymphocyte system (5), is dependent upon energy metabolism but not upon protein synthesis. Previous studies of the effects of colchicine and cytochalasin B upon capping have been contradictory and inconclusive but have involved only mouse cells. Colchicine, vincristine, and vinblastine have been reported to dissolve and inhibit the action of microtubules (28–29) while cytochalasin B appears to affect microfilaments and inhibit cell locomotion (30). In some reports the colchicine derivatives

have shown no effect on mouse lymphocyte capping (5, 8, 25), but one recent study has shown almost complete inhibition of mouse fibroblast capping by colcemid (26). Ault, Karnovsky, and Unanue (15) found no effect of colchicine on human lymphocyte capping but failed to demonstrate capping initially. Cytochalasin B has been reported to inhibit capping partially at a high concentration (5) but not to do so at lower concentrations associated with less cell death (8, 25, 31). Vincristine and vinblastine have not been studied. In this study we have demonstrated, for the first time, a clear-cut inhibitory effect of all three microtubular-inhibiting agents on the capping phenomenon in human lymphocytes as well as glutamic acid reversal of the action of vincristine and vinblastine. Cytochalasin B, in concentrations up to toxic levels, failed to inhibit this process. This suggests that microtubules, not microfilaments, are involved in the directional redistribution of the surface aggregates in normal human lymphocyte membranes and is consistent with data from other laboratories which indicates that colcemid tends to "depolarize" cells and allow continuous random membrane movement (32).

It may be postulated that the directional movement of the aggregated receptors requires fixation to an internal stabilizing structure such as microtubules, which pull these aggregates towards one pole of the cell while displacing the nonbound aggregates to the opposite pole. Both microtubules and actin-like microfilaments have been described in submembrane area of lymphocytes (33) although the latter have been more prominent. In addition, a "colchicine-binding" protein has been described which appears to have considerable importance in maintaining topographic stability of the plasma membrane receptors in mouse lymphocytes, and may play a role in directional receptor movement (34). Since receptor redistribution appears to occur most often in motile cells and often over the uropod, some have suggested that cell motility might be involved in this process (25, 35). However, we have observed the phenomenon in cells floating freely in suspension and in cells without clear uropods. Moreover, cytochalasin B failed to inhibit Ig capping of human lymphocytes, and recent studies of mouse (31) and human lymphocytes [8] have failed to establish a cause and effect relationship between motility and capping. Thus, capping appears to occur in cells with the potential for locomotion though they do not have to be engaged in translational movement at the time.

In this study we have demonstrated that the lymphocytes of patients with CLL do not appear to undergo the same sequence of events as normal cells and fail to exhibit surface Ig capping when the surface is perturbated by divalent anti-Ig antibodies. The first stage of the phenomenon, i.e. linking and aggregation of the receptors into patches, does seem to occur normally or to a greater extent than normal. However, the second phase, i.e., the directional movement towards a single pole of the cell, is absent. Previous studies of concanavalin A receptor mobility in mouse lymphoma cells (36) and anti-HL-A–HL-A complexes of human leukemic lymphocytes (37) have suggested a similar abnormality.

There are a number of possible explanations for the abnormal behavior of CLL cells. First, it is well established that the capping phenomenon requires a bridging lattice work formed by linkages requiring bivalence of the attaching antibody and proximity of the receptor sites (5). Thus, in the mouse lymphocyte, the widely dispersed H-2 sites fail to cap with anti-H-2 unless a secondary antibody is employed while the more closely aligned Ig moieties cap directly with anti-Ig (6). Though the anti-Ig fluorescence of CLL cells was often less than that of normal lymphocytes, CLL cells did form large aggregates or patches on the surface. Since a lack of cross-linking generally results in a continued diffuse distribution (5), a decreased number of Ig molecules cannot provide the entire explanation.

Secondly, Holt, Pal, Catovsky, and Lewis (38) have reported that the cell membrane of CLL lymphocytes is more resistant to osmotic lysis and freeze etching and appears to have increased toughness and resilience suggesting an increased membrane viscosity. On the other hand, the viscosity parameter of membrane movement is also felt to be most intimately involved in the passive first stage of capping which appears to be normal in CLL, thus decreasing the attractiveness of this alternative. In addition, CLL lymphocytes should be metabolically capable of supporting the energy-dependent second stage since previous in vitro studies have demonstrated that CLL and normal lymphocytes are indistinguishable with respect to their energy metabolism (39). Since cap formation has been linked to cell motility by some authors (26, 35), another possibility is that CLL cells are less motile than normal cells and thereby cap less. Schrek (40) has demonstrated, in a qualitative fashion, that CLL lymphocytes exhibit less random motion than normal lymphocytes and we have recently demonstrated in quantitative studies that the motility of CLL lymphocytes is considerably less than that of normal lymphocytes.[8] However, since motility, at least with regard to translational movement, does not appear to be absolutely required for capping, the prerequisite may be a structure involved to some degree in both processes, perhaps via differing pathways. The inhibitor data presented here suggests the probable involvement of microtubules in the capping phenomenon of normal lymphocytes and the possibility that

microtubular function in CLL lymphocytes may be abnormal, although the latter has not been specifically demonstrated. Thus, it may be topographical stability imparted by microtubules (34) that is crucial to intramembranous Ig redistribution and lacking in CLL lymphocytes. Further information in this regard must await more detailed high resolution morphologic studies of CLL and normal lymphocytes.

Surface markers, especially surface membrane Ig, have been extensively employed as indicators of specific lymphocyte populations, and a number of lymphoproliferative disorders have been designated B- or T-cell derived (22). However, some studies of malignant lymphoid cells have shown discordance between markers presumably identifying the same population, thus casting some doubt upon the determination of the origin of such cells (18, 41). Our demonstration of qualitative abnormalities involving Ig components of the CLL lymphocyte membrane should reinforce the danger of considering such abnormal lymphocytes as derived from a specific normal subpopulation of cells solely because of the presence of such surface markers. The role of surface membrane Ig redistribution in the initiation of the immune response is unsettled. Although formation of anti-Ig, Ig complexes on mouse lymphocytes failed to produce stimulation or activation in one report (42), such an effect of receptor-antigen interactions has not been ruled out, and one may speculate that some of the immune deficiencies exhibited by patients with CLL might be related in part to the abnormality in directional redistribution of Ig receptors.

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