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Fast cell membrane displacements in B lymphocytes

Modulation by dihydrocytochalasin B and colchicine

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A novel type of cell membrane movement was characterized in B lymphocytes. Local submicron cell membrane displacements, within the frequency range 0.3–15 Hz, were registered in a murine lymphoma B cell line by a novel optical method based on point dark field microscopy. The cell membrane displacements were measured by monitoring changes in light scattering from very small illuminated areas (0.25 μm^2) at the edge of the cell surface. B lymphocytes manifest a relative change in light scattering of 7.7 ± 1.3% (mean ± SD) which corresponds to cell membrane transverse displacement of 131 ± 22 nm. The confinement of cell membrane displacements to microdomains (≤0.2 μm^2) emerged from the observed dependence of the displacement amplitude on the area size from which it is monitored. Colchicine (1 μM) decreased membrane fluctuations down to a value of 88 ± 14 nm, whereas dihydrocytochalasin B (2 μM) increased the amplitude of membrane displacements up to 184 ± 31 nm. These findings demonstrate the existence of a dynamic mechanical interaction between the cytoskeleton and the cell membrane in the frequency range of 0.3–15 Hz. The modulation of these interactions by the disruption of microfilaments or microtubules is explained in terms of the induced strain changes imposed on the cell membrane.

Cell membrane displacement; Cell membrane fluctuation; Cytoskeleton; Colchicine; Dihydrocytochalasin B; B lymphocyte

1. INTRODUCTION

Cell motility involves different types of cell membrane displacements such as pseudopodal extension, undulations and ruffling that are inherent in the process of cell locomotion [1,2]. Recently, a new type of cell membrane displacement, not associated with cell movement, was reported to occur in erythrocytes, lymphocytes, fibroblasts and cardiomyocytes [3]. This type of cell membrane movement, coined as membrane fluctuation, was found to be a local reversible displacement of the cell membrane in the frequency range of 0.5–20 Hz, possessing an amplitude of 30–340 nm in human erythrocytes and 20–30 nm in the various nucleated cells. These membrane fluctuations may be involved in cellular processes such as cell–substrate adhesion, cell–cell interactions, endo- and exocytosis, and membrane recycling. The cell membrane fluctuations in erythrocytes were found to depend on an MgATP-associated dynamic assembly of the membrane skeleton [4]. The association of the cell cytoskeleton components with the plasma membrane in nucleated cells [5,6] suggests that cytoskeletal elements may be involved in these membrane fluctuations. The present report examines the effect of the disruption of the microfilaments or the microtubules network on cell membrane fluctuations in a murine lymphoma B cell line.

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2. MATERIALS AND METHODS

2.1. *Cell culture*

Murine lymphoma cell line 29M10, an IgM-bearing B cell, which was produced via sorting and cloning of I.29 lymphoma cells [7] was a gift of Dr. I. Zan-Bar. These cells were previously characterized phenotypically as IgM, LyT-1 and B-220 positive cells, which can be stimulated to secrete IgM antibodies and/or to differentiate in isotype switch [7]. The cells were cultured in plastic multiwell plates (Cel-Cult) containing RPMI 1640 (Gibco) medium supplemented with 10% fetal calf serum (Bio-lab), HEPES buffer (10 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (0.05 mM) and L-glutamine (2 mM). Cells were grown at 36.5°C with 5% CO_2 and were reseeded every 2 days so as to maintain them at a logarithmic phase of the growth. The lymphocytes were introduced into the experimental chamber at low density. The experimental chamber consisted of 2 glass coverslips separated by a distance of 100 μm. The volume of the experimental chamber was about 10 μl. The surface of the coverslip, to which the cells adhered, was marked (by a scratch) to enable the return to the same field of view after treating the cells with drugs. After introducing the cells into the chamber they were incubated at 36.5° C for 30 min to achieve cell attachment to the glass surface. After attachment, the cells were washed by 30 μl of medium to remove unattached cells and cell debris, and the chamber was closed by paraffin, ready for the measurement of membrane fluctuations. Dihydrocytochalasin B (Sigma) at a final concentration of 2 μM and colchicine (Sigma) at a final concentration range of 1–20 μM were added to medium after measuring the fluctuation levels in untreated (control) cells. Measurements of the cell membrane fluctuations were carried out for 2 h, following either pre-incubation with dihydrocytochalasin

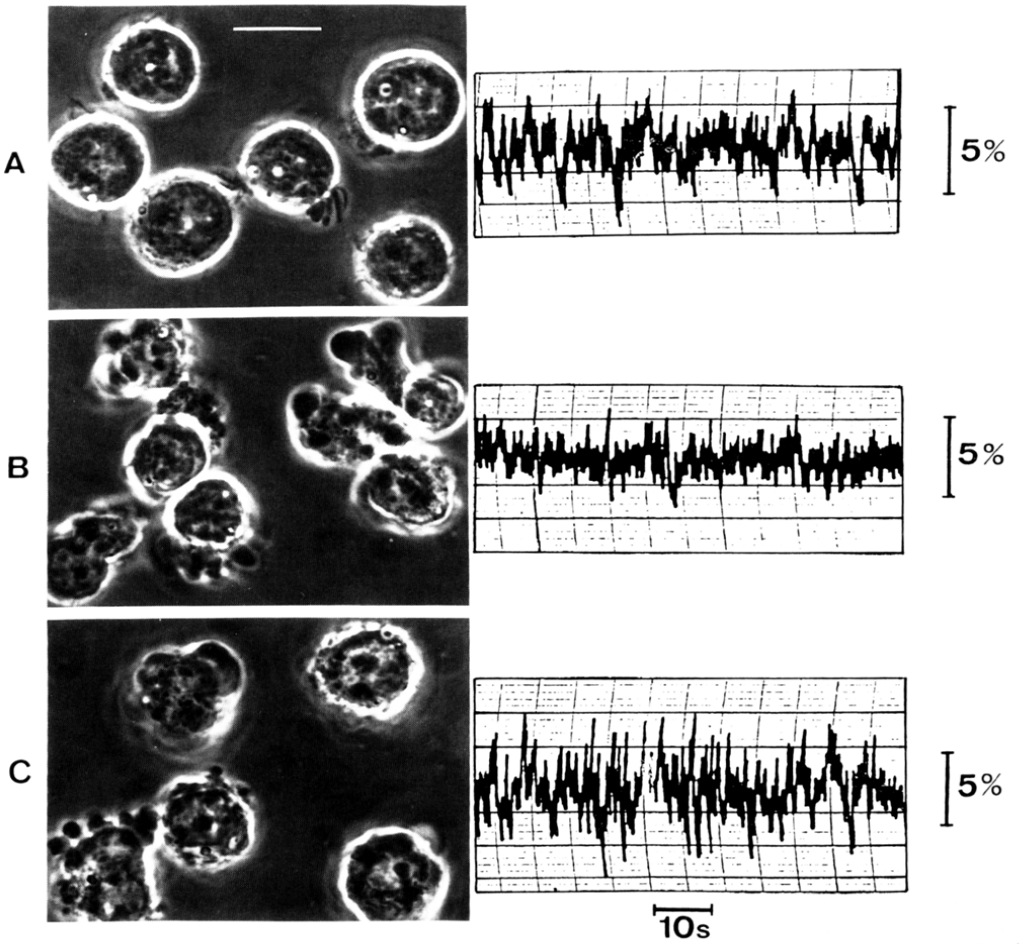

Fig. 1. Influence of colchicine and dihydrocytochalasin B on cell shape and membrane displacements in murine lyumphoma B cell line. (A) control; (B) cells after incubation with 1 μM colchicine for 30 min; (C) cells after incubation for 2 h with 2 μM dihydrocytochalasin B. Left panel (A, B and C), phase-contrast photographs of the living cells attached to the glass surface. Bar, 10 μM. Right panel (A, B and C), typical traces of the relative change of light scattering (δI/I%) from a small illuminated area of 0.25 μm^2 at the cell edge.

B for 2 h or pre-incubation with colchicine for 30 min. In some experiments, cells were incubated with dihydrocytochalasin B at a concentration of 1 μM for 20 h after which they were introduced into the chamber. The pre-incubations with dihydrocytochalasin B or colchicine were carried out at 36.5°C, while the actual measurements of membrane fluctuations were performed at room temperature.

2.2. *Measurement of membrane fluctuations by point dark field microscopy*

Cell membrane displacements were measured by our recently developed point dark field microscopy [3,4]. Briefly, the microscope-based experimental set-up measures local mechanical motion of the cell surface by detecting light scattering changes from a small area of the cell surface. The fluctuation of the light intensity depends on the changes of the membrane area position moving in and out of the focused light spot near the cell edge. A catadioptric objective ×125, N.A. = 1.1 was used as a dark-field condenser to image the field diaphragm on the cell edge, in a way that only scattered light could reach the photodetector. The relative change in light scattering (δI/I%) was obtained by dividing the peak-to-peak change in the AC component of light scattering (δI) by the DC component of light scattering (I) when illuminating a small area of the cell edge. A linear dependence between the relative change in the scattered light (δI/I%) from the cell surface and the amplitude of the cell edge displacement was demonstrated previously [3] by moving the cover-glass, with attached glutaraldehyde fixed cells, by a calibrated vibrator. Linearity of δI/I% with displacement was observed over distances as long as 340 nm (where a relative change in light scattering of 1% corresponds to a displacement of 17 nm [3]). The sensitivity of our experimental set-up is ≤1%. The illuminated area in most experiments was 0.25 μm^2, however in some experiments we studied changes of light scattering from larger areas of 2 μm^2. The level of fluctuations, measured on a single cell, showed some variability with regard to the chosen site of measurement. Therefore, the level of fluctuation was measured in each cell at 4 different sites, 90° apart, and an average value was calculated for each cell.

3. RESULTS

Membrane displacements were measured in 29M10 B lymphocytes which were adhered to a cover-glass (Fig. 1A). About 90% of the cells maintained a round shape (Fig. 1A) for at least 3 h after adhesion to the glass, in the presence of 10% FCS. The amplitude of fluctuation was found to depend on the size of the area measured. Thus, fluctuation amplitude measured from a small area of 0.25 μm^2 yielded fluctuation levels of 8.4 ± 2.5% (n = 15), whereas a smaller fluctuation amplitude of 4.6

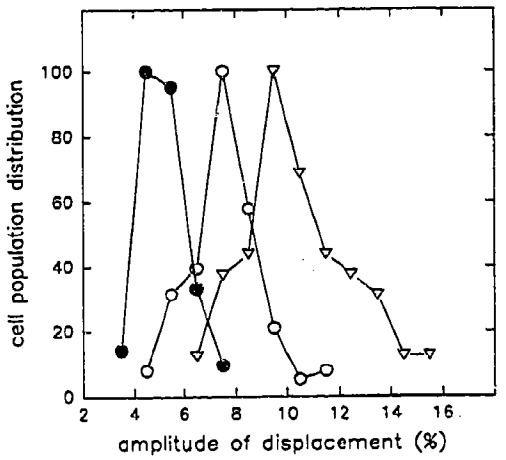

Fig. 2. Distribution of displacement amplitudes (in terms of relative changes in light scattering, $\delta I/I\%$) in the murine lymphoma B cell line. The displacement amplitudes were grouped within a range of 1%. Data are normalized at the most abundant amplitude of displacement (○), control cells (n = 127); (●), cells treated with colchicine (1–20 μM) for 30 min (n = 62); (▽), cells treated with dihydrocytochalasin B (2 μM) for 2 h (n = 68).

± 2.1% (n = 15, $P < 0.0002$) was obtained from an area the size of 2 μm^2. Hence, all further measurements were carried out from an illuminated area of 0.25 μm^2. A histogram of the fluctuation levels in B lymphocytes is shown in Fig. 2. The observed average level of fluctuation was 7.7 ± 1.3% (Table I) which corresponds to a cell membrane transverse displacement of 131 ± 22 nm.

After treatment with colchicine (1 μM, for 30 min) nearly half of the cell population lost its rounded shape and developed large pseudopods that permanently changed their shape (Fig. 1B). Sometimes these cells became rounded but after a certain length of time they again began to develop pseudopods. Treatment with colchicine leads to a decrease in cell membrane fluctuations (Fig. 1B). A histogram of the fluctuation levels in B lymphocytes following treatment with colchicine is shown in Fig. 2. The distribution of membrane fluctuations in colchicine-treated cells was found to be narrower than the corresponding distribution of control cells (Fig. 2). The average fluctuation level of colchicine treated cells was 5.2 ± 0.8% (Table I), which corresponds to a transverse membrane displacement of 88 ± 14 nm. Increasing the colchicine concentration up to 20 μM (for 30 min) did not lead to a further decrease in membrane fluctuations (Table I).

After treatment with dihydrocytochalasin B (2 μM, for 2 h) a large fraction of the cell population is characterized by a nearly rounded shape with uneven edges and permanent slow development and disappearance of small pseudopods (Fig. 1C). The measurement of membrane fluctuations was carried out in areas devoid of these pseudopods. The treatment with dihydrocytochalasin B leads to an increase of fluctuation amplitudes (Fig. 1C). A histogram of fluctuation levels in B lymphocytes following treatment with dihydrocytochalasin B is given in Fig. 2. The observed average level of fluctuation was 10.8 ± 1.8% (Table I) which corresponds to a membrane displacement of 184 ± 31 nm. The same stimulating effect was obtained also after a long pre-incubation (20 h) of the lymphocytes, in culture plate, with dihydrocytochalasin B (Table I). It can be seen that the values of the membrane fluctuations of the dihydrocytochalasin-treated cells hardly overlaps with those of the colchicine-treated cells.

4. DISCUSSION

The local character of cell membrane fluctuations emerges from the observed dependence of the fluctuation amplitude on the area size from which the fluctuation is monitored. Thus, a fluctuation amplitude of 8.4 ± 1.5% was obtained when illuminating a small area of 0.25 μm^2, whereas a lower fluctuation amplitude of 4.6 ± 2.1% was obtained when we illuminated an area of 2 μm^2. This difference may be attributed to the growing interference of light scattering changes due to a larger number of independent sites of incoherent displacements operating when registering larger areas. The increased number of incoherent local fluctuations, when expanding the registered area, leads to the mutual loss in the amount of the changes in the scattered light, yielding an apparent decrease of the registered amplitude. Thus, we may conclude that cell membrane displacements are confined to microdomains (≤0.2 μm^2), excluding the possibility that these fluctuations are due to the movements of large cell regions, such as pseudopods or due to the displacement of the whole cell. The type of cell membrane movement presented in this study (characterized by a frequency range of 0.3–15 Hz) is 2–3 orders of magnitude faster than the cell edge displacement during fibroblast locomotion (characterized by a frequency range of 0.001–0.01 Hz) [2].

The amplitude of cell membrane displacements, measured in a B lymphocyte line derived from a murine lymphoma, is ≈130 nm. This large displacement is roughly 20-fold larger than membrane thickness. Such a displacement involves a large local bending of the cell

Table I

The influence of dihydrocytochalasin B and colchicine on cell membrane displacements in the B lymphocytes

Treatment	Amplitude of displacements ($\delta I/I\%$)	n
Control	7.7 ± 1.3	106
Dihydrocytochalasin B (2 μM, 2 h)	10.8 ± 1.8	41
Dihydrocytochalasin B (1 μM, 20 h)	9.9 ± 1.9	22
Colchicine (1 μM, 30 min)	5.2 ± 0.8	39
Colchicine (5 μM, 30 min)	5.2 ± 0.8	10
Colchicine (20 μM, 30 min)	4.9 ± 0.5	12

All amplitudes of cell membrane displacements in treated cells differ statistically from the control amplitude ($P < 0.001$, Student's t-test).

surface. If one assumes that the bending modulus of the cell membrane is 10^{-12} dyne·cm^{-1} [8], one can calculate that the ratio of energy of bending to thermal energy is extremely large (>1000). This suggests that a metabolic (chemical) source of energy is needed to maintain these cell membrane fluctuations. This large displacement in the B lymphocytes line is 6-fold greater than the 20–30 nm displacement, recently observed in human peripheral blood lymphocytes [3]. This difference may result either from the difference between the species studied or may be attributed to a difference between normal and malignant cells.

The possible involvement of the membrane skeleton in mechanical fluctuations of the cell membrane emerges from a recent study of human red blood cells [4]. However, the potential involvement of the main cytoskeletal elements, microfilaments and microtubules, in a similar type of cell membrane fluctuation in nucleated cells is still unknown. The existence of an association between the cytoskeleton and the cell membrane was previously shown [5,9–11]. These findings lay the foundation for the possible participation of the cytoskeleton in the fluctuation phenomenon. In fact, colchicine, a microtubule-disrupting agent, decreases membrane displacements in B lymphocytes while dihydrocytochalasin B, a microfilament-disrupting agent, increases these fluctuations. This opposite influence of colchicine and cytochalasin was shown already for the capping of the membrane T lymphocyte antigens [10,12].

It was previously shown [13] that an incubation period of 6–8 h with 1 μM colchicine is required for the complete disruption of the microtubule network in lymphocytes. Since our studies show no significant difference in the level of membrane fluctuations between preincubation with 1 μM colchicine for 30 min and 6 h, it may be suggested that only a partial disruption of the microtubules is sufficient to decrease the fluctuation amplitude. Our findings imply that both cytoskeletal elements (microtubules as well as microfilaments) are involved in the mechanical fluctuations of lymphocyte's cell membrane. Although tubulin has been reported to be a significant component of cell membranes, particularly in the neural tissue [14], the direct connection of microtubules with the cell membrane is not yet established. Thus, our results may suggest that the influence of the microtubules on local mechanical fluctuations of the membrane are transduced indirectly, via microtubule-associated cytoskeletal structures.

In contrast to microtubules, actin microfilaments are connected to the cell membrane via multiprotein complexes [6,9,15]. The existence of mechanical constraints in the microfilament cytoskeletal structure may be transmitted to the membrane skeleton. The increase or decrease in the constraint of the membrane skeleton network is expected to lead to a corresponding decrease or increase in membrane fluctuation amplitudes. Thus, the disruption of the cytoskeletal microfilaments by cytochalasin is expected to reduce the constraint on the membrane skeleton. This will lead to an increase in cell membrane fluctuations. When applying colchicine, the destruction of the microtubules, which are connected to intermediate filaments [16,17], was shown to lead to the aggregation of the intermediate filaments into large cables, and consequently to the coiling of these cables around the nucleus [18]. This situation may lead to an increased strain in the membrane skeleton due to the existing association between intermediate filaments and the microfilament cortex [18,19]. This reorganization is expected to result in a decrease in the amplitude of cell membrane fluctuations.

The present study characterizes a novel type of local displacements of the cell membrane in B lymphocytes and describes its modulation by changes in the architecture of the cytoskeleton. These membrane fluctuations, whose physiological function is as yet unknown, may serve as inherent sensors of strain changes imposed on the cell membrane.

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