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ACTIVATION OF HUMAN LYMPHOCYTES BY CONCANAVALIN A OR PURIFIED PROTEIN DERIVATIVE RESULTS IN NO ALTERATION OF FLUORESCENCE POLARIZATION OF LIPID PROBES ALTHOUGH THE ELECTROPHORETIC MOBILITY OF THE CELLS IS CHANGED

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Upon stimulation with either concanavalin A or the tuberculin antigen, purified protein derivative, human peripheral blood lymphocytes, purified on Ficoll-Hypaque, did not exhibit a concomitant lipid fluidity alteration as measured by fluorescence polarization (*P*) of the lipid probe, 1,6-diphenyl-1,3,5-hexatriene (DPH). This result was independent of the incubation period, ranging from 10 min to 72 h. However, a general reduction in polarization value, from $P = 0.287$ (maintained for up to 2 h of incubation) to $P = 0.225$ after 20 h was observed for both experimental and control samples. Moreover, fluorescence polarization studies of the nonpenetrating modified DPH cationic lipid probe, 1-[4′-trimethylaminophenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH), also failed to show any change in lipid fluidity subsequent to a 1–3 h incubation of lymphocytes with concanavalin A. Cell electrophoretic mobility, however, was altered (mean cell mobility increased by 10–15%) in a fast response to stimulation and was observed within several hours of in vitro application of concanavalin A and purified protein derivative. This initial response disappeared with further incubation at 37°C (>3 h) and was followed by a decline of cellular mobility of the concanavalin A-exposed cells after 48 and 72 h of incubation. The unstimulated control cells did not change in mobility as a function of incubation time. The slow decline in mean cell mobility of the experimental cells is believed to be associated with blastogenesis. It is concluded that neither blastogenic transformation nor short term membrane alterations associated with human lymphocyte activation lead to lipid fluidity changes as measured in steady state by the fluorescence polarization of both DPH and TMA-DPH.

Introduction

The expression of alterations in the lymphocyte plasma membrane following cell activation by mitogens or antigens is well established [1–8]. One specific parameter which is of interest in this regard is that of membrane dynamics [9], often referred to as membrane fluidity [10]. This parameter can be measured by the fluorescence polarization method [9–14] which is relatively straightforward and rapid, and therefore could perhaps be used as a sensitive and simple indicator of the activation process [1].

The exact physical interpretation of steady-state fluorescence polarization data is still unclear. Attempts to calculate 'microviscosity' values from them are complicated by the gross assumptions evoked [14]. Moreover, time resolved fluorescence anisotropy decay studies in both cell and artificial membranes revealed that changes in *P* values may primarily reflect alterations in the structural and

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Abbreviations: *P*, fluorescence polarization; DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1-[4′-trimethylaminophenyl]-6-phenyl-1,3,5-hexatriene; NBD-PC, 1-acyl-2-[*N*-(4-nitrobenzo-2-oxa-1,3-diazolyl)aminocaproyl]phosphatidylcholine; F20C, see Ref. 32.

anisotropic nature of the membrane rather than changes only in fluidity [9]. Yet, regardless of the exact interpretation of the observed alterations in membrane dynamics, changes in P values could signal lymphocyte activation.

There are several reports that indicate that this may be the case with polarization changes of a lipid probe after a relatively long-term incubation (i.e. 72 h) of lymphocytes with mitogens [15,16] while other reports have indicated changes only during the rather limited time span of 15–30 min which then disappear after 1 h [5,17]. There also have been conflicting reports in regard to the direction of these changes [18]. Moreover, to add to this confusing picture, the absence of any change in fluorescence polarization values also has been reported recently [19].

In order to investigate the clinical potential for using fluorescence polarization as an indicator of lymphocyte activation, and in order to avoid ambiguities and assumptions about species to species variations, we chose to study human peripheral blood lymphocytes, labelled by the lipophilic probe DPH, and activated by the mitogen, concanavalin A, or the tuberculin antigen, purified protein derivative. The times of incubation covered the entire range of interest from a short as 10 min to as long as 72 h. To the best of our knowledge no such study with human lymphocytes has yet been reported.

We also used a new fluorescent probe, TMA-DPH, which is a modification of DPH. Due to its ionic nature, it anchors to the water-lipid interphase of the cell plasma membrane and hence cannot enter the cell [20]. Rotational rates of DPH and TMA-DPH are not substantially different and studies in model systems revealed that electrostatic interactions of this cationic probe with headgroups of phospholipids do not appear to significantly influence the apparent dynamics of the probe. It retains absorption and fluorescence spectral characteristics similar to DPH, yet its molar extinction is significantly lower than that of DPH while its fluorescence polarization values are higher [20]. Finally besides monitoring fluorescence polarization, we also followed changes in cell electrophoretic mobility after mitogen stimulation in order to assess the relative sensitivity of these two different types of cell surface measurements.

Materials

Tetrahydrofuran (Fischer Scientific Co., Pittsburg, PA) was distilled prior to use. DPH (Aldrich Chemicals, Milwaukee, WI) was used without further purification. Concanavalin A, three times recrystallized, was purchased from Miles Lab., Kankakee, IL. Purified protein derivative was obtained from Connaught Lab., Inc., Swiftwater, PA. Ficoll-Hypaque lymphocyte separation medium was purchased from Litton Bionetics, Kensington, MD. α-Methyl-D-mannoside was purchased from Sigma Chemical Co., St. Louis, MO. TMA-DPH was obtained from Molecular Probes, Plaino, TX.

Methods

Lymphocytes. Human peripheral blood lymphocytes were isolated from heparinized whole blood using the Ficoll-Hypaque density gradient procedure [21]. The isolated lymphocytes were washed three times either with 10% fetal bovine serum in RPMI 1640 with 2 mM L-glutamine or Eagle's minimal essential medium with Hank's salts and L-glutamine (Grand Island Biological Co., Grand Island, NY). Both tissue culture media contained penicillin (10^5 U/l) and streptomycin (100 mg/l).

Determination of purity of lymphocytes. Platelet contamination was approximately 2% in these preparations. Prior to isolation of the mononuclear cells on Ficoll-Hypaque the blood was centrifuged at $170 \times g$ to pellet the leukocytes and to enable removal of the plasma containing the majority of the platelets. Most remaining platelets were lost during incubations and washings prior to fluorescent labeling.

The majority of the monocytes present in the starting preparation was lost (due to their plastic-adherent properties) during the 37°C incubation step with or without mitogen or antigen (see below) resulting in a cellular composition which was on the average, 95% lymphocytes, 5% monocytes (the latter cell type was identified by staining for non-specific esterase).

Incubation of lymphocytes with concanavalin A and purified protein derivative. Incubations were carried out in Falcon tissue culture tubes (catalog. No. 3003, Falcon Plastics, Oxford, CA) in a final

volume of 1 ml. Lymphocytes ($2 \cdot 10^6$/ml) in 10% fetal bovine serum/RPMI 1640 were incubated with concanavalin A (1 μg to 10 μg/ml) for various periods of time at 37°C in 5% CO_2. Control cultures were incubated in the absence of concanavalin A or with concanavalin A in the presence of 0.1 M α-methyl-D-mannoside. The same conditions were used in experiments where lymphocytes were incubated with purified protein derivative (40 μg/ml) or in the case of control cultures, in the absence of antigen. In some of the lymphocyte-concanavalin A experiments the procedure of Inbar and Shinitzky was used [15] where lymphocytes ($2.5 \cdot 10^6$/ml) in 10% fetal bovine serum/Eagle's medium were incubated in 35 mm Falcon plastic petri plates (catalog No. 3001) with or without concanavalin A (1 μg/ml). For short-term incubation of 1 h or less, lymphocytes ($6 \cdot 10^6$/ml) in 0.88% saline were incubated at 37°C without concanavalin A or with concanavalin A (1 μg/ml) in the presence or absence of 0.1 M α-methyl-D-mannoside.

Labeling of lymphocytes by DPH. DPH labeling was performed after incubation with concanavalin A or purified protein derivative. Attempts to label prior to incubation in tissue culture medium resulted in loss of DPH into the medium due to redistribution of this lipid probe [14]. The basic procedure reported by us [9] and by Inbar and Shinitzky [15] was used with slight modifications leading to predominant labeling of the plasma membrane (>95%) with only slight background fluorescence due to DPH distribution within the cell [13,15]. Lymphocytes were washed from tissue culture medium into 0.88% saline containing 0.1% bovine serum albumin. After 10 min they were washed twice with 0.88% saline or 0.15 M KCl. The cells ((4 or 6) $\cdot 10^6$/ml) were then incubated at room temperature with an equal volume of $4 \cdot 10^{-6}$ M DPH in saline (or 0.15 M KCl) for 30 min. A nonlabeled lymphocyte blank was obtained through similar incubation of lymphocytes with an equal volume of 1 : 500 tetrahydrofuran in saline (or 0.15 M KCl). A study of the fluorescence signal intensity as a function of incubation time under the above conditions, revealed that DPH incorporation into the lymphocytes membrane was essentially complete within 30 min. Fluorescence polarization values of lymphocytes labeled under the present conditions were identical to those obtained under the conditions described by Fuchs et al. [11], indicating no DPH-DPH interaction. Fluorescence polarization measurements were taken following two washes with saline, and were carried out with $(4–6) \cdot 10^6$ lymphocytes, at 25°C.

Labeling of lymphocytes by TMA-DPH. The above described procedure for DPH labeling was carried out with the following modifications. The concentration of TMA-DPH labeling solution was $1 \cdot 10^{-5}$ M; TMA-DPH, 10^{-3} M was initially dissolved in 1 : 1 tetrahydrofuran-saline solution. A nonlabeled lymphocyte blank was obtained by incubating lymphocytes with an equal volume of 1 : 200 tetrahydrofuran in saline. Under these conditions, labeling was completed within 15 min and lymphocytes remained viable (>95%) as determined by exclusion of trypan blue.

Fluorescence polarization measurements. These were performed on an MPF-44 spectrofluorimeter (Perkin-Elmer, Norwalk, CT) at 25°C, with $(4–6) \cdot 10^6$ lymphocytes/ml, in a cuvette with a 0.2 mm pathway according to previously described procedures [11,13]. Background scatter obtained from similarly treated unlabeled lymphocytes amounted to ≤5% of the fluorescence intensity at the 430 nm peak emisssion; a 390 nm cutoff filter was employed. Each measurement was repeated at least four times and the mean fluorescence polarization values ±S.D. reported. Lymphocyte viability, determined by exclusion of trypan blue at the end of the measurement, was at least 95%. As is usual, $P = I_{/\!/} - I_{\perp}/I_{/\!/} + I_{\perp}$ where $I_{/\!/}$ and $I_{\perp}$ are the intensities with polarizers parallel and perpendicular, respectively.

[2-^{14}C]Thymidine uptake measurements. 100 μl of lymphocytes ($2 \cdot 10^6$/ml) in 10% fetal bovine serum/Eagle's medium were incubated with or without concanavalin A (1 μg/ml) in microtiter plates with round bottom wells (Falcon Plastics) for 72 h followed by pulse labeling with 0.1 μC [2-^{14}C]-thymidine for 18 additional hours. At least six replicate wells were run per determination. Cells were harvested by a previously described method [22].

Laser Doppler electrophoresis. Cell electrophoretic mobility profiles were determined by the laser Doppler technique [23]. Cell mobility was measured in 0.15 M NaCl using a crossed optics and electrodes as previously described [24,25]. The electrophoretic velocity is detected by the Doppler shift of light scattered from the moving cells in accordance with the equation:

$$\delta\nu = EMK$$

where $\delta\nu$ is the Doppler shift, E is the electric field amplitude along the scattering wave vector K, which in amplitude is just $(4\pi/\lambda)\sin(\theta/2)$ for θ the scattering angle. Spectra from statistically significant cell numbers (>100) can be generated in 1 min or less. The spectra are analogues to mobility profiles of cell populations: faster cells cause higher Doppler frequency shifts than slower cells. The relative amplitude of various spectral peaks is approximately proportional (and exactly so in the case of equal relative scattering amplitudes) to the relative cell numbers. The Doppler spectra were measured with a fast Fourier transform spectrum analyzer (Rockland Systems, Inc., Rockleigh, NJ). Further details of the apparatus and a more thorough description of the technique can be found in several recent reviews [26,28]. For a direct comparison of mobility profiles between experimental and control samples, the temperature and the electric field were kept at a constant value in a given set of measurements. A typical value of the applied electrical field was 25 V/cm and the cuvette temperature was typically at 24°C.

Results

Fig. 1 shows fluorescence polarization values, P, for DPH labeled lymphocytes incubated for up to 72 h. Two trends are evident: firstly, a decline in P values which occurred after 6 h incubation (but not observed for the first 1 or 2 h) to a leveling off value of about 0.225 observed between 20 and 72 h of incubation; secondly, no significant differences in P values between concanavalin A-treated and untreated lymphocytes at any of the incubation time intervals. In addition, these two trends were independent of the culture media; the same results were observed either in RPMI 1640 or Eagle's minimum essential medium. An increase of concanavalin A concentration to 5 μg/ml and even 10 μg/ml also gave the same results. The absence of an effect on P values subsequent to lymphocyte incubation with concanavalin A was not due to the inability of the lymphocytes to undergo transformation in the presence of this mitogen; substantial increases in the uptake of [2-^{14}C]thymidine of 28- and 59-fold, were

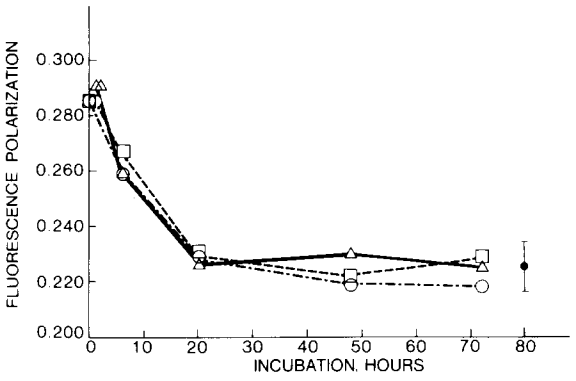

Fig. 1. Fluorescence polarization values of DPH-labeled human lymphocytes incubated for the indicated periods with 1 μg/ml concanavalin A (△———△), without concanavalin A (○· — · — ·○), and with 1 μg/ml concanavalin A and 0.1 M α-methyl-D-mannoside (□- - - - -□). Each time point was repeated on at least two different cell preparations and the average of the mean P values are shown, along with average S.D.

observed for lymphocytes incubated for 72 h with concanavalin A (1 μg/ml) compared to cells incubated in the absence of concanavalin A or in the presence of this mitogen and α-methyl-D-mannoside, respectively.

In view of the absence of a signal after long incubation periods with mitogen, we attempted to determine whether there is a cell membrane modulation occurrring during the first 15–30 min after incubating lymphocytes with concanavalin A which then disappears after further incubation. We performed short term incubation experiments with and without concanavalin A. In these experiments 4 μg/ml concanavalin A were used. Fig. 2 shows that no decline in P values occurred during the first hour of incubation, regardless of the presence or absence of concanavalin A in the incubation medium. Furthermore, P values of lymphocytes exposed to concanavalin A were not significantly different from the controls.

Measurements of cell electrophoretic mobility, summarized in Fig. 3, showed mobility alterations both at short and at long incubation times. After an incubation period of 48 h and greater, a decline in mobility of concanavalin A-treated cells as compared to untreated lymphocytes was observed (see Fig. 4b). This slow change is associated with blastogenesis,

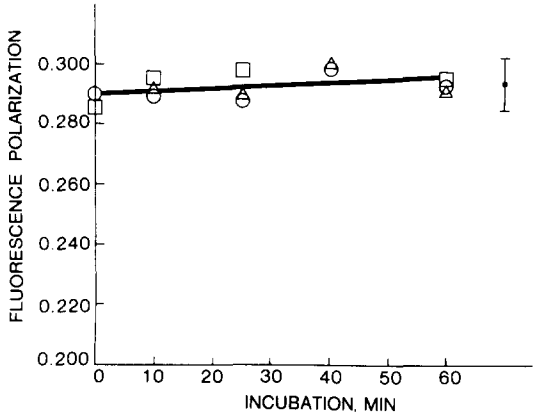

Fig. 2. Fluorescence polarization values of DPH-labeled human lymphocytes incubated for the indicated periods with 4 μg/ml concanavalin A (△), without concanavalin A (○), and with concanavalin A and 0.1 M α-methyl-D-mannoside (□). Each time point was repeated on at least two different cell preparations and the average of the mean *P* values are shown, along with average S.D. The line drawn is based on least square analysis of all data points presented.

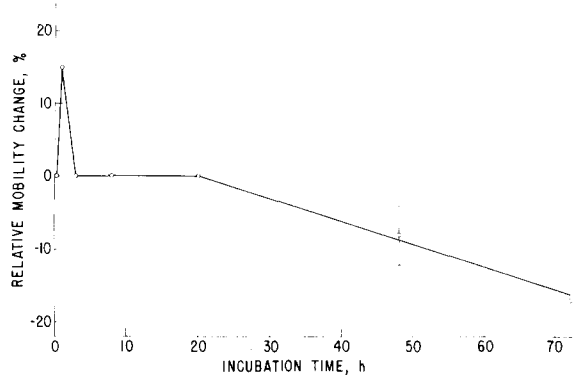

Fig. 3. Mean mobility of concanavalin A-exposed cells relative to concanavalin A-unexposed cells as a function of incubation time. For times of 1 h and less, the cells were kept for an additional 1.5 h at room temperature as discussed in the text prior to mobility measurement. For longer periods the cell mobility was measured shortly after washing and was invariant as to further storage at room temperature. The mobility profile of the control samples does not change (within experimental error) with incubation time. The precision of a single determination is ±4% and is marked on the data points.

as an increase in large blast like cells was observed in the mitogen-treated cell cultures after 48 and 72 h. In contrast to the trend in DPH polarization, the mobility profile of the control culture was unaltered after prolonged incubation.

Fig. 3 also indicates that quite a different membrane phenomenon may be occurring a short time after mitogen binding to the lymphocyte surface. After a brief incubation of 1 h at 37°C, there was no immediate mobility change. However, after storage for an additional 1.5 h at room temperature, the mean mobility of the cells exposed to concanavalin A increased by 10–15% relative to the control cells (see Fig. 4a). If the 37°C incubation step was reduced to 10 and 25 min, this mobility alteration failed to evolve even subsequent to storage at room temperature. Also, if the 37°C incubation step was increased to 3, 6 or 20 h, no difference between concanavalin A and non-concanavalin A treated lymphocytes could be observed, either immediately after cell washing or after room temperature storage.

In view of the delayed effect observed in cell electrophoretic mobility we repeated our lymphocyte-concanavalin A stimulation experiments under similar conditions prior to measuring *P* values. Cells were incubated for 1.5 h at 37°C. After a 1 h storage at room temperature, DPH was added. Following 45 min of room temperature labeling, *P* values were obtained, still showing no effect of lymphocyte activation on lipid fluidity. Cells measured again after washing excess label exhibited the same *P* values, again with no difference between concanavalin

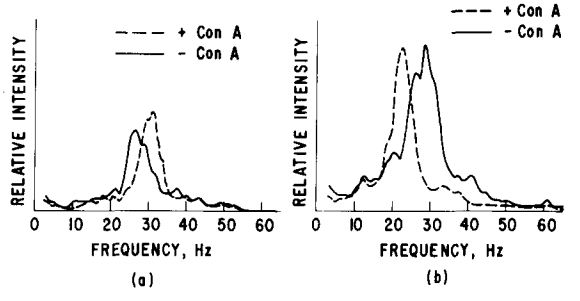

Fig. 4. (a) Doppler spectra of concanavalin A-exposed (- - - - - -) and non-concanavalin A-exposed cells (———) after 1 h incubation at 37°C. (b) Doppler spectra of concanavalin A-exposed cells (- - - - - -) and non-concanavalin A-exposed cells (———) after 72 h of incubation.

TABLE I

CONCANAVALIN A (Con A)-LYMPHOCYTE INTERACTION. EFFECT ON FLUORESCENCE POLARIZATION VALUES, P, OF TMA-DPH

Incubation time (h)	Number of independent experiments for each time point	$\bar{P} \pm$ S.D.	
		+Con A	−Con A
1	3	0.381 ± 0.009	0.387 ± 0.008
1.5	4	0.386 ± 0.008	0.383 ± 0.006
3	1	0.367 ± 0.004	0.375 ± 0.007

A-treated and control lymphocytes. In these experiments P values were also measured with a pulsed laser fluorimeter which reads simultaneously the ratio of the fluorescence intensity in the parallel and perpendicular modes. Despite the higher precision of this set-up (error of ±1%) no difference was observed between concanavalin A-treated and control lymphocytes.

TABLE II

PURIFIED PROTEIN DERIVATIVE-LYMPHOCYTE INTERACTION. EFFECT ON FLUORESCENCE POLARIZATION VALUES, P, OF DPH

Donor type [a]	Incubation time (h)	$P \pm$ S.D.	
		+Purified protein derivative	−Purified protein derivative
(+)	1	0.284 ± 0.003	0.28 ± 0.01
(+)	1.5 [b]	0.277 ± 0.008	n.d. [c]
(+)	1.5 [d]	0.28 ± 0.01	0.280 ± 0.006
(−)	1.5	n.d. [c]	0.276 ± 0.002
(+)	1.5	0.278 ± 0.007	0.276 ± 0.008
(−)	1.5	0.284 ± 0.002	0.285 ± 0.008
(+)	18	0.252 ± 0.009	0.261 ± 0.009

[a] Skin test-positive (+) or skin test-negative (−) to purified protein derivative.

[b] Measured after additional 1.5 h of room temperature storage.

[c] Not determined.

[d] Measured after additional 1.75 h of room temperature storage. Label was introduced after 60 min room temperature storage.

The results obtained using the TMA-DPH probe are shown in Table I. No difference in lipid fluidity of the plasma membrane was exhibited by the activation of human lymphocytes with concanavalin A as compared to unexposed lymphocytes. In order to ensure optimal conditions for measurement comparable to those observed for electrophoretic mobility determinations (Fig. 3) data were taken after the lymphocytes had been stored at room temperature for 1.5 h.

The effect of the tuberculin antigen, purified protein derivative, on lymphocyte fluorescence polarization was also investigated. Table II summarizes the results of these experiments. Regardless of the incubation period, whether as short as one hour, or as long as overnight, no difference between lymphocytes exposed to and unexposed to purified protein derivative was observed. This was the case both for tuberculin-positive and negative donors, as well as for delayed measurements taken after lymphocytes were kept at room temperature for an additional 1.5-h period.

Discussion

Steady-state fluorescence polarization measurements of DPH labeled biological membranes of intact cells provide a measure of the dynamic changes occurring either in fluidity or in the anisotropic nature (i.e. order parameter, degree of restricted motion) of the lipid bilayer core of these membranes (Ref. 7 and references therein). This physical measurement, (although simple and relatively fast), has two major limitations: it provides only an average measure of a heterogeneous system; and it is based on a nonspecific lipophilic probe which although primarily embedded in the plasma membrane, is not exclusively located there, but may penetrate the internal membranes of the cell. However, for the purpose of signaling a change in membrane structure due to the binding of a ligand to a lymphocyte surface, it could be quite suitable, at least in view of earlier literature reports [1,3,10].

In the present study, we found no effect of concanavalin A on the dynamic characteristics of human lymphocyte membranes, as expressed by the measured P values using either DPH or TMA-DPH for incubation periods of up to 72 h (Figs. 1 and 2).

These results are different from those reported by Inbar and Shinitzky [15] who observed a decrease in P values of concanavalin A-treated lymphocytes relative to concanavalin A-untreated cells, using rat lymphocytes isolated from lymph nodes. In addition, an opposite trend in P values with incubation was reported by them: P values increased with incubation. Interestingly though, our initial fluorescence polarization values which were reproducible for all donors examined in the present study, i.e., 0.287 ± 0.006, were similar to those reported by Inbar and Shinitzky, 0.276. As a check on system response to fluidity changes we performed polarization measurements as a function of temperature. The temperature profile of the measured P values in our study followed the standard trend [11–13] yet no difference could be exhibited between concanavalin A-exposed and control human lymphocytes. Moreover, even when measured by the pulsed laser spectrofluorimeter which is of high precision (error is ±1%), due to the simultaneous ratio reading of the parallel and perpendicular polarization modes, the same P values were obtained for both concanavalin A-treated and control cells. The decline in P values with incubation (Fig. 1) could have arisen from changes in cholesterol/phospholipid ratios [10–15] either due to loss of membrane cholesterol or to its reduced cellular biosynthesis after relatively long incubation periods. No such reduction in P values was observed within the first hour of incubation (Fig. 2). The difference in the general change in fluorescence polarization with incubation between our study and that of Inbar and Shinitzky [15] may be due to the difference in lymphocyte source, rat vs human, since otherwise we attempted to use the same experimental protocol.

The observed increase in thymidine uptake and in blastogenesis found with the concanavalin A-treated human lymphocytes as compared to control lymphocytes point to the successful activation of the lymphocytes in the present study. Concomitant with lymphocyte transformation into blast cells there was a decrease in electrophoretic mobility of lymphocytes (Fig. 3). This decrease corresponds well to literature reports on the time course of blastogenesis [29,30].

Lymphocytes incubated for various times with purified protein derivative also did not show any fluidity changes. These results are again in contrast to those obtained by the cell mobility studies (manuscript in preparation) which detected changes in cell surface charge between lymphocytes (obtained from skin tested tuberculin-positive donors) exposed to purified protein derivative and those not exposed to the antigen. We thus conclude from our present study that lipid fluidity, as depicted by P_{DPH}, is not altered by cell activation with either concanavalin A or purified protein derivative, although other membrane characteristics do change, e.g., cell surface charge and cell surface protein migration [31].

Other investigators have described effects of mitogen-binding on the fluidity of lymphocyte membranes. For example, Farber et al. [16] using the fluorescence probe, perylene, reported an increase in fluidity in calf thymus lymphocytes after incubation with concanavalin A for 4 h. Electron spin resonance (ESR) studies of mouse splenic lymphocytes with concanavalin A showed increased fluidity after 15–30 min of incubation which reversed to normal within 60 min [5]. Similar results also were obtained by these investigators for human lymphocytes exposed to phytohemagglutinin. However, Monti et al. [18], using another probe, NBD-PC (1-acyl-2-[*N*-(4-nitrobenzo-2-oxa-1,3-diazolyl)aminocaproyl]phosphatidylcholine, observed an increase in rigidity upon exposure of human lymphocytes to a very high concentration (150 μg/ml) of phytohemagglutinin. In these two latter reports [5,18], the experimental conditions were not completely revealed.

Toyoshima and Osawa [17] studied human peripheral lymphocytes with the lectin, *Wistaria floribunda*, and found effects (by the DPH fluorescence polarization method) similar to that reported by Barnett et al. [5], i.e. a reversible decline in rigidity, with the maximum effect occurring after 15–30 min.

Two additional methods based on lateral mobility of membrane components have been described. Schlessinger et al. [31] reported for the L-6 myoblast-concanavalin A system no effect on lipid motion, as measured by the fluorescence photobleaching technique. Heterogeneous mobility was however reported by them for the lateral mobility of various selectively tagged proteins on the membranes of these cells. Kosower et al. [32], using his membrane mobility agent F20C reported a most profound effect for the thymus-derived mouse lymphocyte-staphylococ-

cal enterotoxin B system. They found after 72 h of incubation a 1000-fold decrease in migration of their mobility agent in transformed cells as compared to normal cells. Recently, Curtain et al. [33] studied human blood lymphocytes by ESR before and after treatment with concanavalin A. They reported a heterogeneous response, expressed in varying order parameter values, for the different membrane regions that were examined. Similar heterogeneity was also reported for the fluorescent probes, DPH and ANS (8-anilino-1-naphthalene sulfonate) pointing out the dynamic differences even betweeen the different membrane lipid domains probed by these two agents [34]. Even when the fatty acid composition of lymphocyte plasma membranes was directly modified by exogeneous fatty acids, there was little effect on the degree of order of the membrane phospholipid fatty acid chains as monitored by the effect on the rotational relaxation time of DPH [35].

From our results and those reported in the literature we conclude:

1. There is no general trend in fluidization, i.e. enhancement in DPH rotational depolarization, occurring in the lipid core of lymphocyte plasma membranes upon interaction with mitogens.

2. The variability in literature reports may arise not only from differences in source of lymphocytes, mitogen used and variation in procedural details, but also, and perhaps more importantly, from the heterogeneity of the membrane structure, even within the lipid core. Thus different probes may sense different regions in the membrane.

3. Steady-state DPH-fluorescence polarization measurements, though rapid and simple, may not serve as an indicator of human peripheral blood lymphocyte activation.

These conclusions bear directly on the important problem of developing a simple and quick assay of lymphocyte activation based on fluorescent probes. Such an indicator would have of course wide clinical application, particularly for detection of malignant growth. A recent attempt at this goal is by measuring changes in the structuredness of the cytoplasmic matrix which is based on fluorochromasia [36–38]. This test has recently proved to be quite controversial [39–41] due to its sensitivity to experimental details and to its inherent complexity of combined membrane transport and cytoplasmic effect [40]. Thus, the present investigation was motivated in part by the need to focus on membrane phenomena alone as suggested earlier by Blakeslee [40]. In view of our present results, i.e. that P_{DPH} cannot depict membrane changes during human lymphocyte activation, other approaches may be called for. These may include time resolved fluorescence measurements as well as specific labeling of various membrane proteins. However, the former involves a significant degree of instrumental complexity and the latter involves the complication of a labeling procedure to a specific component. It may be that the electrophoretic approach to this problem will prove to be the simplest.

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