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STIMULATION OF LYMPHOCYTES BY CONCANAVALIN A

TEMPERATURE-DEPENDENT EFFECT OF FATTY ACID REPLACEMENTS

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Summary

The membrane fatty acyl composition of lymphocytes was altered by growth in lipid-depleted serum containing fatty acid supplements, as well as avidin to block endogenous synthesis of fatty acids. Under these growth conditions over 50% of the total fatty acid in membrane phospholipid were derived from the added fatty acid.

Enrichment of lymphocyte membranes with oleate (*cis* $C_{18:1}$) or elaidate (*trans* $C_{18:1}$) shifted the optimum temperature for mitogenic stimulation by concanavalin A as measured by [^{3}H]thymidine incorporation. These results suggest that the fluidity of the membrane lipid phase plays a role in the process of lymphocyte stimulation by lectins.

Introduction

Plant lectins are carbohydrate-binding proteins which can interact with glycoprotein or glycolipid receptors on the cell surface. Lymphocytes are stimulated in vitro by lectins such as concanavalin A or phytohemagglutinin to undergo DNA synthesis, blast transformation and cell division [1—3]. The stimulation is dependent upon the binding of the lectin to the cell surface [4,5] and requires a minimal time of 12—20 h during which the lectin must be present in order for the cells to be activated irreversibly [6]. However, the nature and function of the lectin receptor sites and the subsequent series of events leading to DNA synthesis and cell division are not yet understood.

There is evidence that the fatty acid composition and the related physical

Abbreviations: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate.

state of the membrane play a role in transport and other membrane-related phenomena [7,8]. It is possible to alter the membrane lipid composition of mammalian cells in culture by the use of lipid-depleted serum supplemented with exogenous fatty acids [9,10]. Using this technique, it was shown that the temperature dependence of agglutination of mouse fibroblasts by concanavalin A and wheat germ agglutinin could be dramatically changed by the substitution of the natural fatty acids with oleate or elaidate which either lowered (oleate) or raised (elaidate) the transition temperature in pure lipid systems [11].

We have investigated the effect of fatty acid replacements on the temperature dependence of lymphocyte stimulation by concanavalin A. The role of fatty acid replacement is discussed in terms of overall temperature-dependent lipid-phase fluidity and possible mechanisms of mitogenic stimulation induced by concanavalin A.

Materials and Methods

Cells. Inguinal and mesenteric lymph nodes from Balb/c mice were used as a source of lymphocytes. To obtain a single cell suspension the nodes were passed through a sieve of stainless steel and further filtered through gauze. The cells were washed three times in medium and adjusted to $3 \cdot 10^6$ cells/ml of culture medium.

Culture media. The growth medium consisted of RPMI 1640 supplemented with 25 mmol HEPES, 2% penicillin-streptomycin (10 000 units/ml) and 10% lipid-depleted serum or untreated foetal calf serum. All sera were heat inactivated for 30 min at 56° C. The cells were cultured in the above RPMI medium containing 10% foetal calf serum, 10% foetal calf serum plus 0.04% avidin, 10% lipid-depleted serum, 10% lipid-depleted serum plus avidin, 10% lipid-depleted serum plus avidin plus 0.005% oleate or 0.005% elaidate. Fatty acids were purchased from Applied Science Laboratories, avidin from Sigma, medium and serum from Gibco.

Mitogenic stimulation. Lymphocytes were incubated in Falcon Micro Test II plates with flat bottoms in 250 µl at a concentration of $3 \cdot 10^5$ cells per well. Concanavalin A, purchased from Miles Yeda Ltd., was further purified in our laboratory by affinity chromatography. Concanavalin A was added to the lymphocyte suspension to yield a final concentration of 0.1, 1, 2.5, 5, 10, 25 µg/ml. After 2 days in culture at a given temperature cells were labelled with 1 µCi of [^{3}H]thymidine per well (Amersham: 5 Ci/mmol) for another 24 h at the same temperature. On the third day, the lymphocytes were harvested by lysis with water and filtration through fiberglass filters and radioactivity was counted with toluene scintillation fluid [12].

Uptake of [^{3}H]thymidine: measurement of total cell-associated radioactivity. $3 \cdot 10^6$ cells grown in different culture media and at different temperatures for varying lengths of time were incubated at the same temperature as before for 5 min with 10 µCi/ml of [^{3}H]thymidine. The incorporation was stopped by adding crushed frozen phosphate-buffered saline, the cells were then washed three times in phosphate-buffered saline, dissolved in 1% sodium dodecyl sulfate and counted in a toluene-Triton X-100 scintillation fluid.

Measurement of soluble pool and acid-precipitable radioactivity. Lympho-

cytes treated as for total cell-associated radioactivity were precipitated with 10% trichloroacetic acid after the third wash. The precipitate was pelleted at 10 000 × *g* for 10 min and 0.1 ml of the supernatant counted in toluene-Triton X-100 scintillation fluid. The pellet was dissolved in 1 M NaOH and an aliquot used to determine the protein content. The remainder was reprecipitated with an appropriate amount of trichloroacetic acid, filtered and counted.

Measurement of leakage. Cells treated as above were suspended in phosphate-buffered saline after the third washing and incubated for 2 h at the same temperature at which they had been stimulated before. The cells were centrifuged and 0.1 ml of the supernatant counted for radioactivity.

Mitotic index. Cells were incubated for 2 days as for mitogenic stimulation and then labelled with [^{3}H]thymidine for 24 h. Afterwards they were washed and fixed in methanol and blown onto slides. The slides were processed for autoradiography using a Kodak nuclear track emulsion. Following exposure for 48 h, the slides were developed, stained with Giemsa and mounted for counting.

Assays. The preparation of lipid-depleted serum was based on the method of Scanu and Edelstein [13] and all manipulations were carried out at −25° C. For the fatty acid analysis lipids were extracted by the method of Bligh and Dyer [14] and processed as described by Horwitz et al. [11]. Protein was determined by the method of Lowry et al. [15] and lipid phosphorus by the method of Ames and Dubin [16]. Viability was checked by trypan blue dye exclusion and cell numbers were determined by Coulter counter.

Results

Incorporation of exogenously supplied fatty acids

The time course and extent of incorporation of exogenously supplied fatty acids into lymphocyte membrane phosphatides was determined at intervals of 12 h over a period of 5 days in culture. Stimulation of lymphocytes by concanavalin A was found to be required for the incorporation of larger amounts of exogenous oleate (*cis* $C_{18:1}$) or elaidate (*trans* $C_{18:1}$). Maximal enrichment of membrane phospholipids with oleate or elaidate was reached in 36 h. These results are in agreement with previous data showing reduced metabolism and low lipid turnover of quiescent lymphocytes [17]. It could be shown that already 10 min after the addition of a mitogenic lectin exogenous [^{14}C]oleate uptake was maximally enhanced. Furthermore the majority of the [^{14}C]oleate was located in the plasma membrane when compared to the endoplasmic reticulum [18], suggesting that the exogenous fatty acids were incorporated quickly and selectively into the plasma membrane upon addition of concanavalin A. The membrane fatty acid composition of stimulated lymphocytes after 3 days in culture is shown in Table I. As can be seen by comparison of control lymphocytes in foetal calf serum with cells incubated in either oleate- or elaidate-supplemented lipid-depleted serum plus avidin, a 3—4-fold enrichment of the exogenous unsaturated octadecanoate ($C_{18:1}$) was obtained, representing as much as 50% of the total membrane fatty acid. Some of the control conditions show also an increased level of unsaturated octadecanoate in their membrane phosphatides, but the doubling of $C_{18:1}$ in foetal

TABLE I

FATTY ACID COMPOSITION OF PHOSPHATIDES FROM LYMPHOCYTES GROWN IN UNMODIFIED OR IN SUPPLEMENTED LIPID-DEPLETED MEDIUM

Cells were stimulated with 3 μg/ml concanavalin A for 3 days in the indicated medium. Fatty acid composition of isolated phosphatides was determined by gas-liquid chromatography as described in Materials and Methods.

Medium supplement	Fatty acid (weight percent of total)							
	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	20 : 0	20 : 4	other
Foetal calf serum	38.9	8.6	23.8	13.2	7.6	1.0	3.3	3.6
Foetal calf serum + avidin	29.1	3.9	24.8	22.2	8.0	1.9	2.6	7.5
Lipid-depleted serum	27.9	6.3	18.0	35.5	8.7	0.3	1.3	2.0
Lipid-depleted serum + avidin	30.0	4.5	14.0	31.6	6.2	3.3	6.6	3.8
Lipid-depleted serum + avidin + oleate	20.0	4.9	18.1	45.5	8.4	0.8	1.1	1.2
Lipid-depleted serum + avidin + elaidate	16.5	6.8	10.1	51.5	8.8	0.9	2.8	2.6

calf serum plus avidin over foetal calf serum seems not to be sufficient to give rise to differences in lectin sensitivity as will be shown below.

Effect of lipid substitution on temperature-dependent stimulation

The maximal responses of lymphocytes to concanavalin A in control medium and fatty acid-supplemented lipid-depleted medium at various incubation temperatures are given in Fig. 1. In non-supplemented lipid-depleted medium containing avidin a decreased capacity to be stimulated was noted throughout the tested temperature range. Thus, the absence of lipid biosynthesis or of suitable exogenous lipid supply appears to restrict not only cell growth [11] for which cell division is required but also earlier steps like S phase. In all other growth conditions tested cells were able either to synthesize their own fatty acids (medium without avidin), or to incorporate fatty acids present in the medium, and showed no significant differences in overall stimulation above 35° C (Fig. 1). Below this temperature, the optimal temperature for stimulation varied depending on growth conditions. Control lymphocytes could be maximally stimulated by concanavalin A at 33° C or above, whereas optimal stimulation of oleate-enriched cells or elaidate-enriched cells occurred already above 30° C and only at 35° C, respectively. No response could be detected below 29.5° C. The effect of lipid modification on lymphocyte stimulation was most pronounced at 30° C, where a 3-fold difference between elaidate- and oleate-enriched cells was observed. Although alternative possibilities must be considered, the data shown in Fig. 1 are consistent with the notion that activation of lymphocytes at low temperatures requires a certain fluidity of the lipid phase (increased by oleate). For the different conditions shown in Fig. 1 maximal stimulation occurred at different mitogen concentrations. The relationship between temperature, concentration of concanavalin A and maximal response for different cell types is shown in Fig. 2. In general, the lower the thermal transition point for maximal stimulation, the lower the concentration

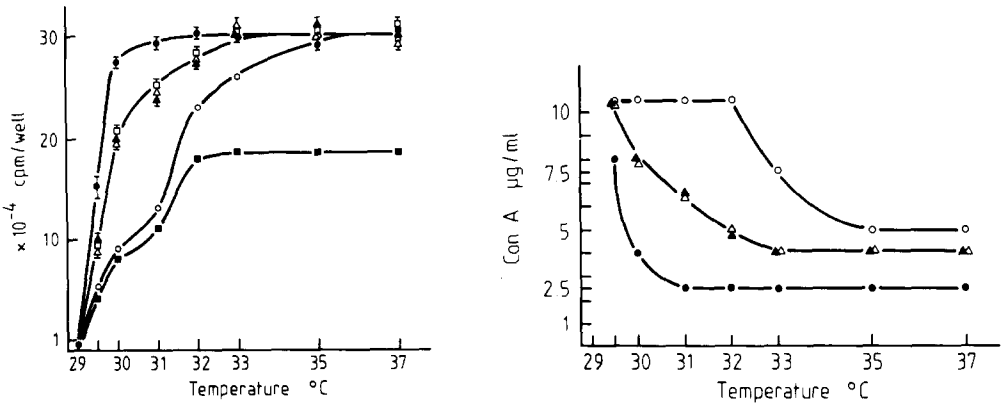

Fig. 1. Effect of membrane fatty acid substitution on concanavalin A stimulation of lymphocytes at various temperatures. The concanavalin A concentration giving rise to maximal stimulation of [^{3}H]-thymidine incorporation was determined at every temperature for the six growth conditions using a range of concanavalin A concentrations between 0.1 and 25 µg/ml. Each point in the diagram represents the mean value of three independent experiments, each performed in triplicate. Lectin was added at day 1 and [^{3}H]thymidine was added 48 h later for an additional 24 h. Experimental conditions: ●, lipid-depleted serum plus oleate plus avidin; ○, lipid-depleted serum plus elaidate plus avidin. Control conditions: △, foetal calf serum; ▲, foetal calf serum plus avidin; □, lipid-depleted serum; ■, lipid-depleted serum plus avidin.

Fig. 2. Effect of temperature and membrane fatty acid substitution on the concanavalin A concentration required for maximal stimulation. Maximal [^{3}H]thymidine incorporation was determined as indicated in Fig. 1. Experimental conditions: ●, lipid-depleted serum plus oleate plus avidin; ○, lipid-depleted serum plus elaidate plus avidin. Control experiments: △, foetal calf serum; ▲, foetal calf serum plus avidin.

of concanavalin A necessary for lymphocyte activation. Such data suggest that membrane fatty acid substitution might influence the interaction between mitogens and surface receptors involved in the activation step.

To rule out the possibility that the above differences in thymidine incorporation were merely due to reduced cell viability after lipid substitution or incubation at low temperature, viability was assayed by trypan blue dye exclusion. By this criterion no differences in viability could be found between the different lymphocyte cultures.

Furthermore, temperature shift experiments were performed to test whether incubation of cells at reduced temperature impairs their subsequent ability to respond to concanavalin A. Cells were kept 2 days at reduced temperature (29 or 31° C) with the agent concanavalin A, then shifted to 37° C for another 2 days after which [^{3}H]thymidine incorporation was measured in comparison to control cells. As shown in Table II, cells kept at 29° C for 2 days and thereafter at 37° C for 3 days showed the same stimulation as the cells kept in culture at 37° C for 3 days only. Thus, cells incubated at reduced temperature appeared to be undamaged and capable of responding fully to concanavalin A at higher temperature.

Additional control experiments tested the possibility that the temperature-dependent differences could reflect the differences in uptake, pool size or leakage of thymidine. Thus we compared the total cell-associated radioactivity, the radioactivity found in the acid-soluble and acid-precipitable material, and the radioactivity leaking out of the cells. These measurements were made at

TABLE II

EFFECT OF INCUBATION AT REDUCED TEMPERATURE ON LYMPHOCYTE STIMULATION

[^{3}H]Thymidine incorporation during the last 24 h of incubation was measured as described in Materials and Methods. Values are expressed as percent of control cells stimulated at 37° C for 5 days.

Medium supplement	Incubation at constant temperatures [a]			Incubation at shifted temperatures [b]
	29°C for 5 days	37°C for 5 days	37°C for 3 days	29°C for 2 days +37°C for 3 days
Foetal calf serum	8	100	192	201
Lipid-depleted serum + avidin + oleate	11	100	188	193
Lipid-depleted serum + avidin + elaidate	3	100	184	200

[a] Cells were stimulated with the optimal concanavalin A concentration at the indicated temperature for 5 or 3 days.

[b] Cells were stimulated with the optimal concanavalin A concentration for 2 days at the lower temperature and for 3 days at 37°C.

12-h intervals over a period of 5 days at 29, 31, 33 and 37° C. Table III shows a representative set of data obtained after 2.5 days at 31° C (time and temperature corresponding to the conditions in Fig. 1 where a 3-fold difference in thymidine incorporation was seen). At 31° C, the total cell-associated radioactivity is higher for control cells than for cells with an altered membrane fatty acid composition, suggesting possible differences in uptake, pool size, or both. However, the radioactivity in acid-precipitable material shows the expected ratio of incorporation at 31° C: greater for oleate-enriched cells than for elaidate-enriched cells. In no case could differences in uptake, radioactive pool size or leakage over the temperature and time range studied (data not shown) account for the observed differences in [^{3}H]thymidine incorporation into acid-precipitable material.

TABLE III

COMPARISON OF [^{3}H]THYMIDINE UPTAKE, INCORPORATION AND LEAKAGE BY CONTROL-LYMPHOCYTES AND FATTY ACID-SUBSTITUTED LYMPHOCYTES

Cultures containing $3 \cdot 10^6$ cells were stimulated with 3 μg/ml concanavalin A at 31° C for 2.5 days and subsequently incubated with 10 μCi/ml [^{3}H]thymidine for 5 min. Incorporation was stopped by addition of frozen phosphate-buffered saline and cells were washed three times with ice-cold phosphate-buffered saline. For measurement of leakage an aliquot of each culture was incubated at 31° C for an additional 2 h. Acid-soluble and -precipitable radioactivity and cellular protein were determined as described in Materials and Methods. All radioactivity values refer to 1 μg of cell protein. The radioactivity associated with the washed cells is taken as 100%.

Medium supplement	Cell-associated radioactivity		Acid soluble		Acid precipitable		Leakage	
	cpm	%	cpm	%	cpm	%	cpm	%
Foetal calf serum	521	100	478	92	43	8	107	21
Lipid-depleted serum + avidin + oleate	147	100	93	63	54	37	34	23
Lipid-depleted serum + avidin + elaidate	164	100	139	84	25	15	40	24

Our conclusion that differences in thymidine incorporation reflected different numbers of cells being stimulated rather than different amounts of radioactivity incorporated per cell was further supported by autoradiography of cells grown and labelled under different culture conditions. As shown in Table IV, the proportion of radiolabelled cells in relation to culture temperature and fatty acid substitution showed the same pattern as described in Fig. 1. Oleate-enriched cultures have a higher percentage of cells incorporating thymidine than control cultures or elaidate cultures at 31°C. The amount of incorporated radioactivity per nucleus, judged by grain counts, appeared roughly constant throughout the different growth conditions.

Finally, to test whether the cells were intrinsically capable of dividing at low temperature, once they were stimulated, lymphocytes were first incubated at a temperature at which activation could occur, then shifted to reduced temperatures. We observed that cells which had been stimulated at 37°C and shifted down to 29°C were able to continue dividing for at least 2 days following the shift (data not shown). This finding is in agreement with reports that division of fibroblasts can take place above 15°C [19—21].

In another experiment, cells were incubated with optimal concanavalin A concentrations in foetal calf serum for 24 h in a way that all the responsive cells had a chance to undergo blast transformation. Then the hapten sugar α-methylmannoside was added for 30 min, the cells were washed and incubated at different temperatures in different growth media for an additional 24 h, after which [^{3}H]thymidine was added. Incorporation of radioactivity into DNA was assayed 24 h later as described in Materials and Methods.

Table V shows the results of this experiment in comparison to the control conditions, where the cells were stimulated and grown under constant conditions throughout the experiment. As expected the control groups exhibit a marked difference in stimulation depending on the type of fatty acid and the temperature. The experimental groups show roughly the same degree of stimulation under all culture conditions, although at reduced temperature cells divide a little more slowly. There is no difference between the fatty acid-supplemented cultures, suggesting that the temperature and the fatty acids in

TABLE IV

EFFECT OF TEMPERATURE AND MEMBRANE FATTY ACID SUBSTITUTION ON LYMPHOCYTE STIMULATION AS DETERMINED BY AUTORADIOGRAPHY

Cells were stimulated with 3 μg/ml concanavalin A for 3 days under the growth conditions indicated above. 1 μCi [^{3}H]thymidine was added at 48 h and 24 h later cells were processed for autoradiography as described in Materials and Methods. Each value represents the average of four randomly selected fields containing 200 or more cells, with a maximal standard deviation of ±2%. Results are expressed as percent of cells labelled.

Medium supplement	Temperatures			
	37°C	33°C	31°C	29°C
Foetal calf serum	22.1	20.9	14.2	2.9
Lipid-depleted serum + avidin + oleate	21.1	21.4	20.9	6.8
Lipid-depleted serum + avidin + elaidate	20.6	15.2	9.2	0.6

TABLE V

EFFECT OF CONCANAVALIN A STIMULATION OF CELLS AT 37°C IN FOETAL CALF SERUM FOLLOWED BY REMOVAL OF CONCANAVALIN A AND SHIFT TO MODIFIED CULTURE CONDITIONS

Temperature	Medium supplement	Control cultures [a] ($\times 10^{-4}$ cpm/well)	Experimental cultures [b] ($\times 10^{-4}$ cpm/well)
37°C	Foetal calf serum	27.8	16.3
	Lipid-depleted serum + avidin + oleate	27.3	16.8
	Lipid-depleted serum + avidin + elaidate	27.6	17.1
33°C	Foetal calf serum	28.3	16.1
	Lipid-depleted serum + avidin + oleate	28.1	16.8
	Lipid-depleted serum + avidin + elaidate	21.1	16.8
31°C	Foetal calf serum	19.3	10.9
	Lipid-depleted serum + avidin + oleate	25.9	11.0
	Lipid-depleted serum + avidin + elaidate	10.6	11.6
29°C	Foetal calf serum	0.4	11.6
	Lipid-depleted serum + avidin + oleate	0.4	11.4
	Lipid-depleted serum + avidin + elaidate	0.4	10.6

[a] Control lymphocytes were stimulated with the optimal concanavalin A concentration for 3 days under the conditions specified.

[b] Cells were stimulated with the optimal concanavalin A concentration for 1 day at 37°C in foetal calf serum, treated with 25 mmol α-methylmannoside for 30 min, then washed and incubated for 2 days under the culture conditions indicated. [^{3}H]Thymidine incorporation was measured during the last 24 h in culture.

the growth medium present during concanavalin A triggering are not only relevant for late metabolic steps in DNA synthesis and cell division but also at earlier steps, namely during the activation period of the mitogenic response.

A fatty acid analysis by gas-liquid chromatography showed that at the time thymidine incorporation was measured, the shifted cells exhibited the same fatty acid composition as the control cells in the same altered medium. The differences between the control and experimental cultures at 37°C could be due either to an inhibitory effect of the hapten sugar or more likely to a continuous stimulation of the cells in the control cultures, where the mitogen concanavalin A was not removed.

We also incubated cells in different growth media at 29°C and shifted them up. to 37°C at various times after the additions of the lectin (Table VI). Cells incubated at 29°C with concanavalin A did not show any mitogenic stimulation, but if they were brought up to 37°C, they always showed an increase in thymidine incorporation 12 h after the shift, regardless of the time they had been incubated at 29°C in the presence of concanavalin A. This experiment suggests that the block must be among the early events of lymphocyte stimulation, for after release the induction period is the same as for control conditions.

If the same experiment was done with preincubation of the cells at 31°C

TABLE VI

TIME FOR HALF-MAXIMAL STIMULATION IN SHIFT-UP EXPERIMENTS

Cultures were incubated for 12 h or 24 h at 29°C or 31°C and then shifted to 37°C for varying lengths of time. 1 μCi of [^{3}H]thymidine was added 6 h before harvesting. Maximal stimulation was 200 000 cpm ^{3}H incorporated per culture.

Temperature	Lipid-depleted serum + oleate +avidin	Foetal calf-serum	Lipid-depleted serum + elaidate + avidin	Temperature	Lipid-depleted serum + oleate + avidin	Foetal calf-serum	Lipid-depleted serum + elaidate + avidin
37°C	27	28	28.5				
12 h 29°C—37°C	36.5	38	39	12 h 31°C—37°C	35	39	40
24 h 29°C—37°C	45.5	47	48	24 h 31°C—37°C	39	44	47
29°C	∞	∞	∞	31°C	40	51	∞

instead of 29° C, then elaidate-enriched cells showed an increase in thymidine incorporation 12 h after the temperature shift to 37° C, whereas oleate-enriched cells incorporated thymidine at 31° C. These results further support the hypothesis that the temperature and the growth medium at the time of mitogenic stimulation determine the following events.

Discussion

Lymphocytes grown in lipid-depleted serum plus avidin are able to incorporate exogenously supplied fatty acids, but the added fatty acid is never the only one found in the membrane phosphatides. The cellular pool, contaminants in the medium, incomplete inhibition of de novo synthesis and subsequent metabolism of the incorporated fatty acids may all contribute to the ultimate lipid composition. Nevertheless, the experimental procedure used in this study allows considerable manipulation of the membrane fatty acid composition and provides a means to study the effects of membrane lipid alterations on cellular physiology.

We have demonstrated that lymphocytes grown in lipid-depleted medium supplemented with fatty acids differ in their responsiveness to the mitogen concanavalin A, depending on the supplied fatty acid. Lymphocytes enriched in oleate show a lowered thermal transition temperature for concanavalin A activation in comparison to control cells, whereas elaidate-enriched cells show an increased thermal transition temperature. Incorporation of oleate, a monounsaturated fatty acid with a *cis* double bond and a melting temperature of 13.4° C in a homogeneous phase, lowers the thermal transition temperature of membranes. Elaidate, the *trans* isomer of oleate, has a much higher melting point (45° C) and correspondingly raises the liquid-cristalline phase transition temperature of membranes [7,8]. If concanavalin A stimulation is dependent on lipid phase fluidity, replacement of *cis*- by *trans*-unsatutrated fatty acids would be expected to increase the transition temperature of the mitogenic response, as we in fact observed. Such an effect was most striking at 30° C, where a 3-fold difference in lymphocyte stimulation was found between oleate- and elaidate-enriched cells. The proposed differences in lipid phase fluidity dependent on fatty acid replacement are supported by preliminary spin-labelling studies (data not shown).

A series of control experiments indicated that the effects of membrane fatty acid replacement on lymphocyte stimulation were not due to altered cell viability, capacity to divide, or differences in thymidine uptake, but rather reflect the cells' capacity to undergo blast transformation in response to mitogen. Furthermore, the direction of the changes observed in transition temperatures for lymphocyte stimulation are consistent with analogous studies on bacterial transport systems [7,8], lectin agglutination of lipid-substituted fibroblasts [11], as well as mouse lymphocyte stimulation after fusion with lipid vesicles [22].

One can imagine several mechanisms by which alteration of the surface membrane lipid composition could affect mitogenic stimulation by lectins. Binding of concanavalin A leads to a selective redistribution of its receptors, a phenomenon which could clearly be related to membrane fluidity [23–25]. To

what degree cross-linking and redistribution is necessary can be questioned, however based on a recent report that monovalent concanavalin A was shown to stimulate [26]. Nevertheless, it has been proposed that a decrease in membrane viscosity would favor the segregation of proteins surrounded by phospholipids containing saturated fatty acids and the exclusion of those proteins associated with more fluid lipids [17]. Localized alteration or redistribution of surface membrane lipids might also affect the interaction of membrane proteins with underlying structures [27]. Among the earliest recorded events during mitogenic stimulation are changes in permeability to ions and small molecules [5]. Such changes could result from altered phospholipid metabolism, especially of plasma membrane fatty acids. It has been reported that mitogenic stimulation activates plasma membrane-bound fatty acyltransferases [28]. This activation leads to a redistribution of fatty acids in the phospholipids. There is also evidence for increased phospholipid turnover, with enhancement of oleate incorporation into lecithins of the plasma membrane [18] after stimulation. Further evidence obtained from spin labelling studies suggest that mitogenic lectins induce a rapid increase in membrane fluidity [29], although this finding has been disputed [30]. Remodeling of membrane lipids by acyltransferases could clearly be dependent on the available fatty acid substrates, the temperature, or both. Such possibilities, and the unresolved role of early membrane lipid alterations in the process of lymphocyte activation, are topics for further investigation.

If the cell division initiating signal depends on lateral mobility and clustering of receptors, lipid involvement would be an obvious postulate. Triggering could, however, also result from conformational changes of a single receptor protein or transmembrane protein complex which would not necessarily involve lipids. The results presented in this paper, however, indicate that lipids are involved in the stimulation process. Whether the altered lipid composition affects the lipid pool, the intracellular membranes or more likely, the physiological state of the plasma membrane a participation of lipids must be considered and included in every model of stimulation.

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