CHOLESTEROL DEPLETION FROM BIOMEMBRANES OF MURINE LYMPHOCYTES AND HUMAN TONSIL LYMPHOCYTES

Transformation effects

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1. Introduction

Cholesterol is an important constituent of a number of biomembranes. The role of cholesterol has been actively studied and effects on fluidity as well as lipid–cholesterol interactions have been investigated using a range of physical techniques [1]. There appears to be general agreement concerning the effect of cholesterol on lipid fluidity but some confusion concerning effects on enzyme activity [2]. We have shown that the interaction of liposomes of dipalmitoylphosphatidylcholine (DPPC) with rat thymocytes resulted in the reduction of membrane-cholesterol levels and an altered mode of action of the Na^+/K^+ pump [3].

Here we report our studies of modulating the cholesterol content of lymphocyte membranes. Depletion of cholesterol levels results in reduction of cell transformation to blast cells.

2. Materials and methods

Lymphocytes were isolated by application of a tissue homogenate to a Lymphoprep ficoll gradient [3]. Liposomes of dipalmitoylphosphatidylcholine (DPPC) were prepared by sonication [3]. Cholesterol depletion was achieved by incubation of 10^9 cells with DPPC liposomes (10 mg) in RPMI 1640 medium in the presence of bovine serum albumin (BSA, 200 mg) [3]. BSA was added as it tended to prevent cell clumping. Control cells were incubated in RPMI 1640 with no liposomes of BSA. Upon cholesterol depletion, all incubations were carried out in RPMI 1640 (Gibco) supplemented with 10% foetal calf serum (FCS).

Cell viability was checked by the trypan blue exclusion test. No bacterial or fibroblast contamination was found.

Lipids were analysed by the method in [4]. Fatty acid methyl esters were prepared according to [5]. Cholesterol was extracted, using cholestane as internal standard, by the method in [6]. The samples were methylated in a sealed tube with a 2-fold molar excess of N_1O_1-bis (trimethylsilyl)trifluoroacetamide for 18 h at 20°C, dried and redissolved in cyclohexane before GLC analysis.

Transformation was measured by the uptake of [2-^{14}C]thymidine (8 μCi/ml) at 37°C into the cells after 48 h incubation with various concentrations of lectin (0–10 μg/ml).

Concanavalin A (con A) and phytohaemagglutinin (PHA) were obtained from Sigma, [2-^{14}C]thymidine (50 μCi/ml) was from the Radiochemical Centre, Amersham and DPPC from Fluka.

3. Results

3.1. *Cholesterol depletion*

The cholesterol levels of rat thymocytes incubated with sonicated DPPC liposomes in the presence of BSA was reduced by 70% compared to controls (table 1). Similar depletion occurred with the human tonsil lymphocytes (table 2).

3.2. *Cholesterol reincorporation*

The possibility of cholesterol reincorporation into

Table 1
Cholesterol content of control and cholesterol-depleted rat thymocytes

Cholesterol content	Controls	Cholesterol depleted cells [DPPC (+200 mg BSA)]
nmol/10^7 cells ±SEM	6.6 ± 0.11 (23)	2.12 ± 0.143 (6)

Rat thymocytes were incubated with DPPC liposomes at 37°C for 18 h, washed extensively and assayed for their cholesterol content. The number of independent experiments is shown in brackets. DPPC is dipalmitoylphosphatidylcholine

Table 2
Cholesterol content of control and cholesterol-depleted human tonsil lymphocytes

Cholesterol content	Controls	Cholesterol depleted cells [DPPC (+200 mg BSA)]
nmol/10^7 cells ±SEM	8.25 ± 1.13 (4)	1.67 ± 1.08 (4)

Human tonsil lymphocytes were incubated with DPPC liposomes at 37°C for 18 h, washed extensively and assayed for their cholesterol content. The number of independent experiments is shown in brackets

a cholesterol-depleted rat thymocyte membrane was investigated by incubating the cholesterol depleted cell with 10% FCS for 5 h. 10% FCS contains 1.5 mg/ml bound cholesterol [7]. No cholesterol reincorporation was observed (table 3).

3.3. *Transformation*

Fig.1 shows the effect of cholesterol depletion of rat thymocytes and their activation by con A. Cholesterol depleted cells showed a much reduced uptake of [2-^{14}C] thymidine compared to the control cells. Fig.2 shows results of cholesterol depletion of human tonsil lymphocytes and their activation by PHA. Cholesterol-depleted tonsil lymphocytes also showed a reduced ability to transform compared to controls upon lectin stimulation.

4. Discussion

Our studies show that cholesterol depletion markedly inhibits cell transformation. This result is in accord with the conclusions of other workers who used different methods for cholesterol depletion. Thus treatment of L-cells with 25-hydroxycholesterol, correlating with a decrease in sterol concentration and a decrease in the molar ratio of sterol to phospholipid in the plasma membrane [8,9] resulted in decreased cell division.

Cholesterol depletion of thymocytes modified the activity of the Na^+/K^+ pump and increased the flux of Na^+ and K^+ down their chemical gradients [3] the net result being a decrease in K^+ (internal) and a slight increase in Na^+.

Normally when cells are stimulated to divide (e.g., by PHA treatment) an increase in $^{42}K^+$ is reported [10,11]. In [12] membranes of activated cells become highly leaky to cations like K^+, yet no net change in $[K^+]$ was observed 24 h after the PHA treatment. PHA thus appears to produce a marked increase in K^+ permeability resulting in an increase in the passive leak of K^+ from the cell and an increase in the active K^+ influx. This rapid K^+ influx does not increase cellular K^+ but is required to maintain normal intracellular K^+ levels which have been shown to be essential for specific cellular synthetic pathways necessary for subsequent cell stimulation [13]. During blastogenesis, the cell swells in volume by

Table 3
Cholesterol content of control, DPPC liposome-treated and DPPC liposome-treated thymocytes which have been subsequently incubated with foetal calf serum

Cholesterol content	Controls	Liposome-treated cells	
		DPPC liposomes	DPPC liposomes followed by incubation with 10% FCS
nmol/10^7 cells ±SEM	8.8 ± 0.53 (6)	5.5 ± 0.76 (5)	4.8 ± 1.07 (5)

Measurements were made as described in the text. The number of independent experiments are shown in brackets

at least 1 order of magnitude, thus the influx:efflux ratio must rise to ensure that internal K^+ levels remain constant. Alterations in cholesterol levels of the thymocyte by effecting the Na^+/K^+ pump may thus alter the delicate Na^+ and K^+ levels essential for transformation.

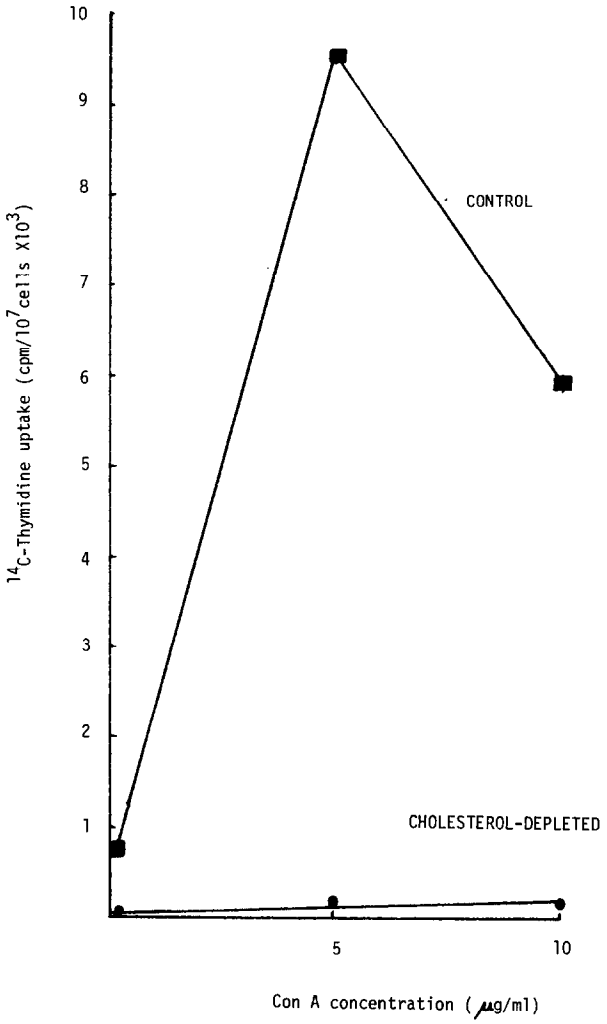

Fig.1. Transformation of control and cholesterol-depleted rat thymocytes (10^7 cells/ml). Cholesterol depletion occurred after incubation for 18 h with DPPC liposomes in RPMI 1640 (+BSA) at 37°C. Control cells were similarly incubated but with no liposomes or BSA added. The cells were harvested, resuspended in 10% FCS in RPMI 1640 to 10^7 cells/ml and incubated with various concentrations of con A. Transformation of the rat thymocytes was measured by the uptake of [^{14}C]thymidine (Amersham, 8 μCi/ml). Label (50 μl) was added after 48 h incubation with con A. The cells were harvested 18 h after addition of [^{14}C]thymidine, and prepared for scintillation counting.

There is also evidence that adenyl cyclase activity is altered by changes in cholesterol levels [14]. It has been suggested that [15] cell division and activation require decreased cAMP levels. If cholesterol does alter the activity of adenyl cyclase, or if adenyl cyclase and the Na^+/K^+ pump both compete for the same ATP pool then an increased pump activity associated with cholesterol depletion could cause the

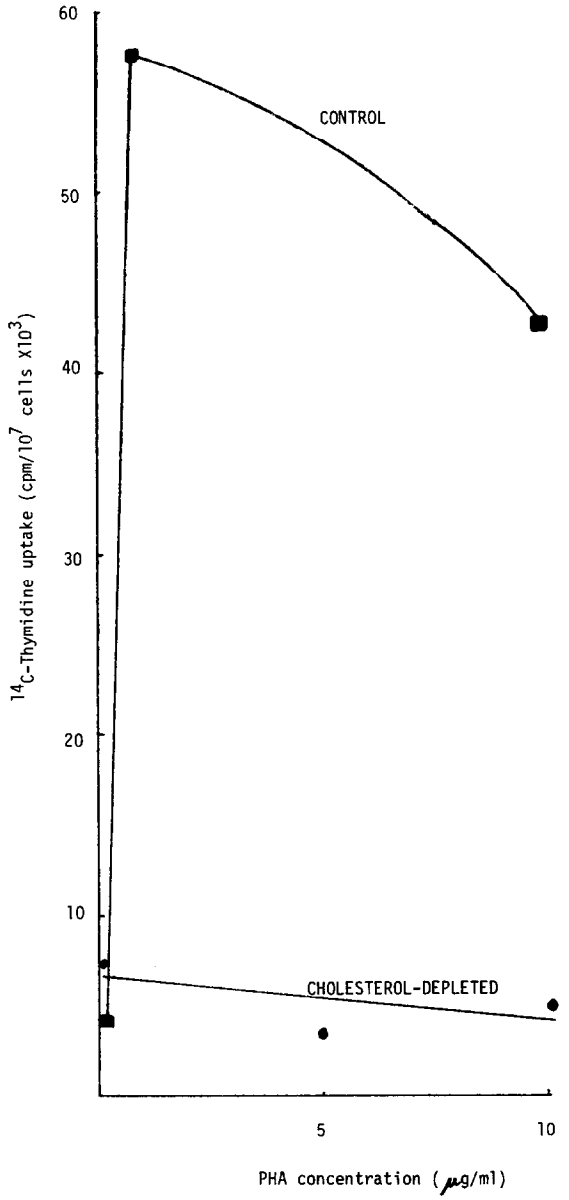

Fig.2. Transformation of control and cholesterol-depleted human tonsil lymphocytes (10^7 cells/ml). Experimental conditions were as in fig.1, except the mitogen used was PHA.

cAMP synthesis to be reduced [16]. This would cause transformation to decrease.

Under normal physiological conditions, the cell maintains a constant level of cholesterol and normal activation and division ensues. However, there are some situations where cholesterol levels may be altered, for example familial hypercholesterolaemia, cancer or nutritional disorders. An altered cell division rate could then possibly occur.

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