ENRICHMENT OF LYMPHOCYTES WITH CHOLESTEROL AND ITS EFFECT ON LYMPHOCYTE ACTIVATION

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1. Introduction

Since the development of the concept of the cell membrane as a mosaic of lipid bilayer and globular protein [1,2], there has been much interest in the relationship between cell properties associated with the plasma membrane and the composition and physical state of the membrane lipids. One such property is the activation of lymphocytes to blast cells by antigens and lectins such as concanavalin A (Con. A) or phytohaemagglutinin. This has been associated with the movement of receptor molecules which are probably partially embedded in the plasma membrane. These receptors are normally randomly distributed over the surface, but rapidly aggregate into patches and caps after antigen or lectin binding [3–6]. This does not occur at low temperatures where the decreased fluidity of the membrane lipids presumably restricts the motion of proteins embedded in them. As Con. A does not have to cross the cell membrane to stimulate the lymphocyte [7], information must be transmitted across it and it is for this reason that the movement of surface-bound activating molecules is of interest.

We have studied, amongst other processes, the effects of increasing the cholesterol content of the lymphocyte upon its activation by lectins. The sterol content of the cells was raised by incubation with liposomes of high cholesterol content. The extra cholesterol was present, at least in part, in the plasma membrane. Such treatment does not alter the ability of the cells to bind Con. A but it markedly suppresses their subsequent activation to blast cells.

2. Materials and methods

Egg phosphatidylcholine preparation, lipid extraction, estimation of phospholipid, cholesterol and protein were as previously described [8,9]. Con. A was obtained from Sigma, London and labelled with [^{3}H]acetic anhydride by the method of Miller and Great [10] to give a specific activity of 3.45 μCi/mg. [^{3}H]acetic anhydride and [^{3}H]thymidine were from the Radiochemical Centre, Amersham.

All incubations were carried out in Eagle's Medium (B.D.H., Poole) supplemented with 10% calf serum. Lymphocytes were prepared from fresh bovine mesenteric lymph nodes [11] and the plasma membrane fraction isolated from them by the method of Lopes et al. [12]. This fraction showed per mg of protein a 6–10-fold increase in cholesterol, ouabain-sensitive ATPase and 5^1-nucleotidase over the original cells. Liposomes containing 1.3–2 mol of cholesterol per mol of phosphatidylcholine [13] were used to enrich the lymphocytes with cholesterol. For this, about 10^7 cells were incubated overnight at room temperature with about 15 μg of liposome phospholipid per ml of medium.

Lymphocyte activation was determined by measuring the uptake of [^{3}H]thymidine after 48 hr incubation with various concentrations of Con. A [14]. Cell viability was assessed from Trypan Blue exclusion 1 min after addition of a 1% solution of the dye.

3. Results and discussion

3.1. *Enrichment of cells and their membranes*

The bovine lymphocytes used in these experiments

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contained about 22 μg of cholesterol/mg protein. After incubation overnight with the liposomes, this was increased by 14 to 88% in a series of experiments. Cells heavily enriched (i.e. > 50%) with cholesterol showed no activation by Con. A (or by phytohaemagglutinin). However, it was noted that although these cells still excluded the vital stain, their basal level of thymidine uptake was much reduced. This could be because the extra sterol inhibits thymidine uptake, as cholesterol does decrease membrane permeability [15]. On the other hand, it seemed possible that the heavy loading with cholesterol was damaging to the cells so the results given here are only for enrichments of < 50%. Shinitzky and Inbar [16] have also recently used liposomes to enrich lymphocytes with cholesterol, but their preparations contained smaller proportions of cholesterol and the incubation period was much shorter. Thus their cells were enriched with cholesterol by only about 16%. A similar increase was obtained here with liposomes of low sterol content.

Two experiments were carried out in which the plasma membrane fraction was isolated after incubation of lymphocytes with the liposomes. The results given in table 1 show that an appreciable proportion at least of the extra sterol taken up by the lymphocytes is in the plasma membrane.

This fraction probably contains the bulk of the cholesterol of animal cells and its content is increased in the two experiments by 40–46%. The cholesterol/phospholipid ratio of the plasma membrane fraction from the untreated lymphocytes is like that obtained by Demus from human lymphocytes [17] but is lower than that obtained from pig lymphocytes [11, 18]. This may be a species difference or could be because the latter provides a more pure preparation.

Table 1
Enrichment of lymphocyte plasma membrane fraction with cholesterol

Origin of plasma membrane fraction	Cholesterol content (mol/mol phospholipid) 1	2
Control cells	0.63	0.67
Cells incubated with liposomes	0.88	0.98

Control cells were incubated overnight in medium only. Test cells in experiments 1 and 2 were incubated with liposome preparations containing 1.8 and 2.0 mol of sterol/mol phospholipid respectively.

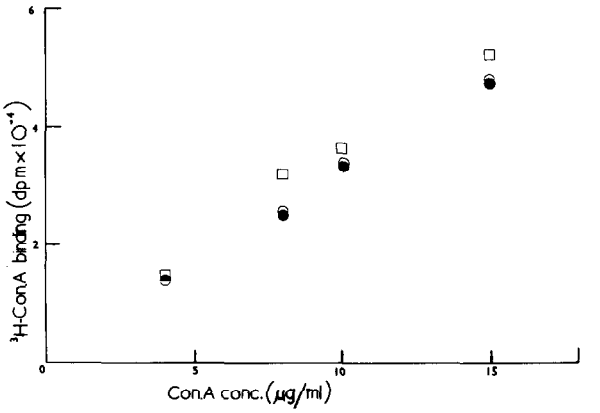

Fig.1. Uptake of [^{3}H]Con. A by normal and cholesterol-enriched lymphocytes. The cells (5 × 10^5/ml) were incubated for 24 hr at room temperature with [^{3}H]Con. A and washed three times before counting. (○) control cells containing 0.46 mol cholesterol/mol phospholipid; (□), cells preincubated overnight with liposomes containing 1.0 mol cholesterol/mol phospholipid which increased the sterol content by 14.4%; (●)cells preincubated overnight with liposomes containing 1.32 mol cholesterol/mol phospholipid, which increased the sterol content by 47.4%.

3.2. *Binding of Con. A by normal and cholesterol-enriched lymphocytes*

The uptake of [^{3}H] labelled Con. A by normal lymphocytes and by those enriched with 14% and 47% cholesterol is shown in Fig.1. It can be seen that over the range of Con. A concentrations used in this investigation, both groups of cholesterol-enriched cells bind the same amount of lectin as the controls. Thus any differences in the effects of Con. A upon these cells must result from events occurring after the initial binding by the cells.

3.3. *Lymphocyte activation*

Fig.2 shows the results of cholesterol enrichment of lymphocytes upon their activation by Con. A. Untreated lymphocytes show the expected stimulation of thymidine uptake with the maximum effect (375%) at 8.5 μgCon. A/ml and declining at higher concentrations. Cells whose cholesterol content had been increased by 14.4% showed a much smaller stimulation (44%) and the optimum concentration of

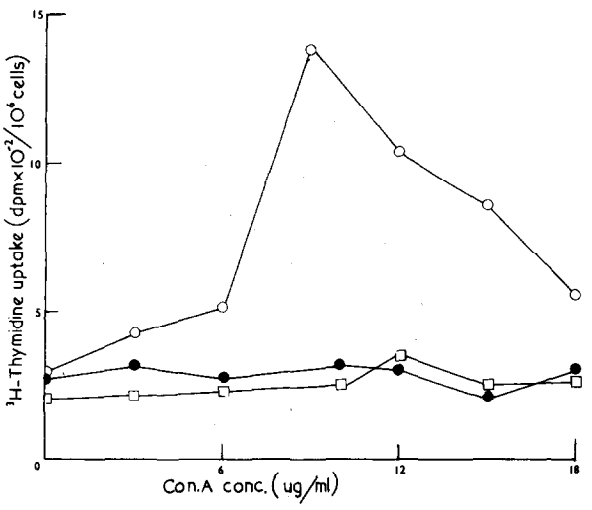

Fig.2. Activation of normal and cholesterol-enriched lymphocytes by Con. A. The cells are those described in fig.1 with the same symbols used for each group.

Con. A was rather higher (12 μg/ml). The experiment was performed in duplicate. The measured thymidine uptakes at each Con. A concentration agreed within 7.5%, so the stimulation, although small, appears to be genuine. When the cell cholesterol concentration had been raised by 47%, no stimulation of thymidine uptake took place at any of the Con. A concentrations used. In similar experiments, there was no activation of the lymphocytes at up to 24 μg Con. A/ml.

As the basal levels of thymidine uptake were similar for all three groups of cells, the effects were not caused by non-specific cell destruction. As a further check, the oxygen uptakes of samples of each group were measured in a Gilson respirometer and again no significant differences were found. It remains to be established whether the small stimulation shown by the cells whose cholesterol content was increased by 14.4% represents a normal response by a small group of cells which had resisted cholesterol-enrichment or a smaller response by all enriched cells.

A general property of cholesterol is to make fluid regions of membranes more rigid [19] and Shinitzky and Inbar [16] have demonstrated that the microviscosity of the lymphocyte plasma membrane is increased by enrichment with cholesterol. This will decrease the rate at which transmembrane receptor glycoproteins can move within the membrane and this may be necessary for the transmission of information across the structure [20]. However, this movement may not be directly related to cell activation [21] and cholesterol has other effects upon membranes. Its insertion into a membrane will cause membrane expansion which could result in separation of non-mobile membrane components involved in the response. The lowering of permeability mentioned above could also be critical since Ca^{2+} uptake has been shown to be an important factor in the activation of lymphocytes by phytohaemagglutinin [22]. We have found (unpublished results) that activation by this lectin is also suppressed by cholesterol enrichment. The more general importance of membrane sterol levels to cell function has been emphasised by recent work on malignant cells [23].

Acknowledgements

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