Cholesterol as a Bioregulator in the Development and Inhibition of Leukemia
(malignant transformation/membrane microviscosity/lipid layers/normal leucocytes/leukemic cells/blood serum)

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ABSTRACT  Leukemia in mice and humans is accompanied by a marked deficiency of unesterified cholesterol in the surface membrane of leukemic cells as compared to normal leucocytes. This deficiency induces a significant reduction in their membrane microviscosity. Since cholesterol in the cell surface membrane is exchangeable with cholesterol in the serum lipoproteins, concomitant to the cellular deficiency of cholesterol, the average level of cholesterol in the blood serum of leukemic patients is substantially below the average normal level. Based on these observations and the effect of membrane microviscosity on biological functions, a working hypothesis that describes the role of cholesterol in the development and inhibition of leukemia is suggested. This hypothesis can also account for the effect of cholesterol and membrane microviscosity on various other cellular activities of leukocytes.

Recent studies in which attempts have been made to elucidate the molecular basis of control mechanisms that determine normal and abnormal cell growth and differentiation, have led to the notion that malignant transformation of normal cells is associated with changes in the dynamic behavior of the cell surface membrane. In accord with this notion, studies on the interrelations between membrane dynamics and biological functions of cells should be primarily based on the composition and the distribution pattern of the cell surface lipid components, which determine to a major extent the degree of lateral and rotational mobility of functional receptor sites (1–6), and the gross plasticity properties of the cell surface membrane (7, 8). These two dynamic features presumably play an important part in controlling the amplitude of regulation signals that are conducted from the cell periphery to its interior, and are closely related to the structural organization of the surface membrane components.

A major lipid constituent of many biological membranes, in particular the surface membrane of mammalian cells, is cholesterol (9, 10). In recent years, with the improvement of physical techniques, such as electron spin resonance (11, 12), nuclear magnetic resonance (13), and fluorescence (14–16), it was shown that under physiological conditions the presence of cholesterol markedly increases the rigidity and the degree of order of lipid bilayers (11–16). The cholesterol level in the cell surface lipid core may thus be implemented to regulate normal and abnormal cell growth and differentiation processes.

With the aid of a highly sensitive and accurate technique (14–16) based on fluorescence polarization analysis of the fluorescent hydrocarbon probe 1,6-diphenyl 1,3,5-hexatriene (16) when embedded in the surface membrane lipid core of intact cells (17, 18), we have recently shown that the microviscosity in the surface membrane lipid layer of normal lymphocytes from rats and mice is almost twice that of malignant lymphoma cells from mice (18). This difference in dynamics originates from a significant cholesterol deficiency, of about half the normal amount, in the surface membrane of the malignant lymphoma cells (18). An analogous difference in microviscosity in the surface membrane was also observed in our laboratory in human lymphocytes from normal donors and from leukemic patients (M. Inbar, M. Shinitzky, and H. Ben-Bassat, submitted for publication). This observation correlates well with the cellular lipid composition of human normal lymphocytes and leukemic cells reported by Gottfried (19, 20). His data reveal that the most pronounced difference in lipid composition between these cells is the markedly lower cholesterol level in the leukemic cells. Moreover, the cholesterol deficiency in acute lymphoblastic leukemic cells is greater than in chronic lymphatic leukemic cells (Table 1). An analogous difference is also found when the lipid composition of isolated plasma membranes from human normal lymphocytes (21) is compared with that of human chronic lymphatic leukemic cells (22) (Table 1). It should be noted that, in addition to cholesterol, the presence of sphingomyelin increases the microviscosity of lipid layers (16), and leukemic cells are deficient in this lipid as well (19).

Introduction of exogenous cholesterol into the surface membrane of intact cells can be performed with (mole/mole) lecithin–cholesterol liposomes (23–25), and in the malignant lymphoma cells from mice it resulted in an increase in the microviscosity to a value characteristic of mouse or rat normal lymphocytes (18). Extraction of native cholesterol from the surface membrane can be performed with lecithin liposomes (23–25), and in normal lymphocytes from mice or rats this resulted in a decrease in the microviscosity to a value similar to that found in the mouse lymphoma cells (18). The changes induced in vitro in the cholesterol content and in its corresponding microviscosity are practically reversible for both cell types, and, in principle, the treated cells can assume any of these characteristics between the limits presented by the values found in the normal lymphocytes and the lymphoma cells (18). The facile exchange in vitro of cholesterol between liposomes and the intact surface membrane of the two cell types indicates that similar translocations of cholesterol can also occur in vivo between the cell surface membranes and the serum lipoproteins and their cholesterol reservoir, as was shown for erythrocytes (26). Indeed, our results show that the lower level of cholesterol in the surface membrane of human leukemic cells and a lower level of cholesterol in the blood serum as compared to normals are concomitant, which is in line with the hypocholesterolemia observed in leukemic patients by others (27, 28) (Table 1). Furthermore, Burstein and Fine (29) have reported that leukemic patients have a deficiency of about 50% in their β-lipoprotein, the protein that is responsible for most cholesterol exchange processes between the serum and cells in the blood (30, 31).
Unesterified cholesterol is believed to be present almost exclusively in the cell surface membrane (9, 32), and to a first approximation the membrane microviscosity depends only on its cholesterol to phospholipid molar ratio (Fig. 1). By virtue of controlling the microviscosity of the cell surface membrane, the level of cholesterol may play a major role in determining biological activities of normal and leukemic cells. This hypothesis is supported by the following experiments, which were recently conducted in our laboratory. Malignant lymphoma cells from mice, which have accumulated cholesterol up to the level found in mouse normal lymphocytes, were inoculated intraperitoneally into mice and the development of ascites tumor was followed. The results have shown that with 10⁶ treated lymphoma cells per animal, full survival was observed up to 90 days after inoculation, whereas with 10⁸ untreated lymphoma cells all infected mice died within 30 days after inoculation (33). On the other hand, by activation of normal lymphocytes in vitro with mitogens such as concanavalin A, it was found that the microviscosity of the surface membrane lipid core decreases upon activation and that cholesterol-deficient lymphocytes are more susceptible to activation than native lymphocytes (in preparation). Although not much is known about the distribution pattern of cholesterol in the cell surface membrane, it can be suggested that cholesterol may act on two distinct levels in determining biological activities of normal and leukemic cells: (a) on the molecular level it may determine the amplitude of, or it may even turn on or shut off specific biochemical signals that depend on the degree of lateral or rotational mobility of specific receptor sites that are embedded in the cell surface lipid core; and (b) on the cellular level it may create a gross tensile force over the cell periphery that will act as a mechanical barrier in cell growth, differentiation, and division processes.

The outline given above strongly suggests that the cholesterol level in the cell surface membrane lipid core and in the blood serum can be utilized to regulate processes that are associated with the development of leukemia. Based on the data which are given in Table 1 and Fig. 1, we would like to suggest a general working hypothesis, which is summarized in Fig. 2. In normally regulated cases, where the molar ratio of cholesterol to phospholipids in the cell surface membrane and in the blood serum are within the normal range, the rates of cholesterol biosynthesis and exchange (35) can preserve and regulate the normal degree of membrane microviscosity that is characteristic for normal growth and development. However, when the ratio of cellular cholesterol to phospholipids and the level of unesterified cholesterol in the serum are below the normal ranges, the rate of cholesterol accumulation in the cell surface membrane can decrease to a critical level where the membrane microviscosity will retain a reduced value that is characteristic of leukemic cells. The reduced levels of membrane cholesterol and microviscosity can, therefore, result either from genetic defects in the rates of cellular biosynthesis of cholesterol and phospholipids during the development and maturation of normal leukocytes or from metabolic defects in

**TABLE 1. Cholesterol levels in intact normal lymphocytes and leukemic cells, in isolated plasma membranes, and in human blood serum of normal and leukemic patients**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cholesterol</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental animals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal lymphocytes (rats or mice)</td>
<td>0.88</td>
<td>(19, 20)</td>
</tr>
<tr>
<td>Malignant lymphoma cells (mice)</td>
<td>0.75</td>
<td>(21)</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>0.38</td>
<td>(19, 20)</td>
</tr>
<tr>
<td>Intact cells</td>
<td>0.38</td>
<td>(22)</td>
</tr>
<tr>
<td>Myeloproliferative diseases</td>
<td>0.25</td>
<td>(19, 20)</td>
</tr>
<tr>
<td>Allergies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Human blood serum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>220 ± 60</td>
<td>(34, b)</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>140 ± 40</td>
<td>(28, b)</td>
</tr>
<tr>
<td>Myeloid metaplasia</td>
<td>120 ± 50</td>
<td>(28)</td>
</tr>
<tr>
<td>Chronic granulocytic leukemia</td>
<td>120 ± 50</td>
<td>(28)</td>
</tr>
</tbody>
</table>

* Values are ± SEM.

**Fig. 1.** The dependence of membrane microviscosity on the molar ratio of cholesterol to phospholipids as determined by fluorescence polarization analysis of the fluorescent probe, 1,6-diphenyl 1,3,5-hexatriene, embedded in lipid regions (16–18).

- Surface membranes of normal lymphocytes from rats or mice (18); O, surface membranes of malignant lymphoma cells from mice (18); A, surface membranes of human normal lymphocytes; △, surface membranes of human chronic lymphatic leukemic cells; □, liposomes of lecithin-sphingomyelin (2:1 mole/mole); •, human erythrocyte ghost membranes. Bars indicate SEM.

The described relation can be expressed by the empirical equation, log q = 0.17 + 0.6·c/p, where q is the microviscosity at 25° in poises (18) and c/p is the molar ratio of cholesterol to phospholipids.

* M. Inbar, M. Shinitzky, and H. Ben-Bassat, submitted for publication.

b Y. Barenholz and M. Shinitzky, unpublished results.

the sources that supply unesterified cholesterol to the lymph and the blood stream.

The mechanism suggested above can be of a more general nature and may take part in determination and regulation of various physiological functions of leukocytes. A controlled reduction of cholesterol level in normal leukocytes may thus sensitize immune response processes or phagocytic activity above the threshold level beyond which malignant transformation and the development of leukemia may occur. On the other hand, a controlled enrichment of cellular cholesterol in leukemia cells may prevent the development of latent leukemia and may hopefully remit leukemia in its active form.