Cholesterol and Its Biosynthesis in Normal and Malignant Lymphocytes

H. J. Heiniger

The Jackson Laboratory, Bar Harbor, Maine, 04609

Abstract

The presently available information on the role and function of cholesterol in plasma membranes of mammalian cells has been reviewed extensively before. This paper restricts itself to briefly summarize some observations gathered in our laboratory and in those of other investigators which directly address themselves to the regulation of the biosynthesis of cholesterol and its possible significance in immunocompetent cells. It is suggested that de novo synthesis of cholesterol represents a critical metabolic step for proliferation and, possibly also, differentiation of lymphoid cells such as cytotoxic T-cells. De novo synthesis of cholesterol is regulated specifically by a feedback mechanism involving oxygenated derivatives of cholesterol. Some of these oxidation products are known to be generated spontaneously from cholesterol, which in itself is not affecting the activity of the rate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC 1.1.1.34). At present, it is not known to what extent oxidized derivatives of cholesterol contaminate the human diet. If they do, their effects on the immune system may be significant, and further investigations on such minor yet very potent and naturally occurring compounds in the diet are needed to understand the etiology of several human diseases. These compounds may also be of therapeutic value in the treatment of some malignant disorders.

It was widely believed that the synthesis of cholesterol in any tissue is regulated by a feedback mechanism of cholesterol itself on the rate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC 1.1.1.34). This concept was mainly based on studies in which the administration of dietary "cholesterol" inhibited its de novo synthesis. It was assumed that the cholesterol added to the diet was and remained chemically pure during the course of the experiments (for review, see Refs. 5 and 14). Recent studies have revealed, however, that chemically pure cholesterol was noninhibitory and that contaminants present in laboratory samples of cholesterol are potent inhibitors of sterol synthesis. These contaminants are now identified as oxygenated derivatives of cholesterol which can be easily generated by autoxidation of cholesterol in the presence of oxygen. A series of investigations on established tissue culture cells (L-cells, CHO cells) demonstrated that the inhibition of de novo synthesis of cholesterol leads to a depression of DNA synthesis and cell growth, alterations in Na⁺-K⁺ flux, depression of endocytosis, changes in the binding of ligands to cell surface receptors, and decreased cell to cell and cell to substrate adhesiveness and partial loss of microvilli (5, 8, 12).

Role of Cholesterol Synthesis in T-Lymphocytes

Based on these observations, we investigated the synthesis of cholesterol, its regulation and role for the functions of immunocompetent cells, specifically T-lymphocytes, in mammals. It was found that lymphocytes, when stimulated to proliferate and differentiate, synthesize cholesterol de novo from acetate (3). When lymphocytes were exposed to phytohemagglutinin (3), they entered into a discrete period of sterol synthesis prior to entering DNA synthesis (S phase). It could be clearly demonstrated that, when the cells were exposed to inhibitors of cholesterol synthesis, the lymphocytes would not enter the S phase, and thus DNA synthesis was abolished. Our studies have since been confirmed and extended by several other laboratories (1, 13) using a similar approach. It was also established that direct inhibition of DNA synthesis by antimetabolites (such as hydroxyurea) did not affect sterol synthesis (13).

Whether the requirement for cholesterol synthesis in proliferating lymphocytes is directly related to the synthesis of new membrane material or whether it constitutes an obligatory biochemical step upon which DNA synthesis depends in some yet unidentified way is not yet settled. Recent studies by Chen (1) present evidence for the first of the alternatives mentioned above, as do our own findings in primary mixed lymphocyte cultures (6) where the effects of the inhibitors of cholesterol synthesis could be overcome by addition of relatively large amounts of exogenous cholesterol or by mevalonic acid, the product of 3-hydroxy-3-methylglutaryl coenzyme A reductase. The latter observation [which was also made in established tissue cell cultures (12)] is of particular interest because it rules out a nonspecific cytotoxic effect of the oxygenated sterols; such an effect could obviously not be prevented and overcome by mevalonic acid. It may be mentioned in this context that the production of CO₂ from acetate (intermediate metabolism) and the synthesis of fatty acids, precursors of membrane-bound phospholipids, were not affected by the inhibitors of cholesterol synthesis (3).

At first hand, the statement that inhibition of proliferation interferes with the functions of immunocompetent cells appears rather trivial since it is clear that any specific clone of such cells requires considerable amplification through proliferation before it can manifest itself. Our observation, in allogeneic one-way mixed lymphocyte cultures, that sterol synthesis proceeded beyond the onset of DNA synthesis precipitated the question of whether a requirement for sterol synthesis exists in T-lymphocytes to achieve their differentiated cytotoxic state concomitant to its requirement for the proliferation cycle. To separate these 2 distinct requirements, we used 11-day-old primary mixed lymphocyte cultures which were restimulated by allogeneic cells or supernatant from secondary mixed lympho-

1 Presented at the Workshop on Fat and Cancer, December 10 to 12, 1979, Bethesda, Md. This research is supported by Grant CA 19305 from the NIH.
2 The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care.
cytotoxic titer in such restimulated cultures rises more than 10-fold during the first 24 hr in the absence of DNA synthesis or even in the presence of inhibitors of DNA synthesis such as arabinoside-C. We found that, shortly after restimulation, the mixed lymphocyte cultures exhibit a significant increase in cholesterol synthesis; concomitantly, their cytotoxic titers increased. Twenty-four hr after restimulation, cytotoxic titers were up 10-fold and sterol synthesis increased 20-fold. This took place in the presence or absence of arabinoside-C. When oxygenated derivatives of cholesterol, such as 25-hydroxycholesterol, were added at concentrations of 0.5 to 3 μg/ml to the culture at the moment of restimulation, the cytotoxic titers not only did not increase at all, but the residual titers of Day 11 were lost (8). The effects of the oxygenated derivatives were prevented by adding cholesterol in 5% bovine serum albumin solution (1 to 3 mg/ml) or mevalonic acid (1 mg/ml) to the cultures. Recently, Humphries and McConnell (10) obtained analogous results by analyzing the secretion of antibody by B-cells (9).

The addition of oxygenated derivatives of cholesterol (i.e., 25-hydroxycholesterol) to the 3-hr cytotoxicity test (6) (51Cr release) itself, even in high doses (>200 μg/ml), did not affect it. Also, the effect of the inhibitors of sterol synthesis did not seem to be primarily related to the effector-target cell binding (doublet formation) since addition of phytohemagglutinin as a ligand does not restore cytotoxicity, although this manipulation is known to overcome allogeneic mismatching or can be used to demonstrate polyclonal activation of cytotoxic T-lymphocytes. Surprisingly, inhibition of sterol synthesis in the target cells did not result in differences in cytotoxic titers, although the spontaneous release (background) of 51Cr by the target cells was higher.

The most likely interpretation of all these data at present is that cholesterol represents a critical membrane component of cytotoxic T-cells and that its de novo synthesis plays a significant role in these cells, possibly even in the presence of lipoprotein-bound serum cholesterol.

Sterol Synthesis in Leukemic Cells in Mice and Humans

Spleen cells, specifically lymphocytes, from naive unstimulated animals synthesize very little cholesterol. In contrast to this, leukemic cell populations display an unusually high rate of sterol synthesis (2). Circulating leukemic parablasts showed up to a 300-fold increase in sterol synthesis as compared to normal circulating peripheral leukocytes in AKR/J mice (2). Fatty acid synthesis was elevated 22-fold, and CO2 production was elevated 16-fold.

Leukocytes from normal healthy human beings also displayed a very low synthesis of cholesterol from acetate, whereas cells from patients suffering from leukemia showed a massive increase in sterol synthesis (7). Cells from acute myelocytic leukemias displayed a 50-fold increase in sterol synthesis as compared to controls; in lymphosarcoma, the increase was 30-fold; and in acute histiocytocytic leukemia and acute lymphocytic leukemia, the increase was 15- to 20-fold (7). Interestingly enough, in a case of chronic lymphocytic leukemia, no increase was observed. The significance of the increased sterol synthesis in leukemia is unknown at present. It is not correlated to serum levels of cholesterol in these patients, nor does there seem to be any significant changes in cholesterol/phospholipid ratios in the plasma membranes (12) despite that, claims have been made that the membranes of leukemic cells are more "fluid" (13). It appears that these observations were related to cell environment and transplantation rather than to the leukemic state per se (15); (for a detailed discussion of this subject, see Ref. 5).

Leukemic cells were as sensitive to inhibition of their sterol synthesis by oxygenated sterols (i.e., 25-hydroxycholesterol) as were normal cells (4), suggesting no major abnormality in their feedback mechanism.

Conclusion

The bulk of recent experiments suggest that the synthesis of cholesterol and its regulation is of critical significance for the proliferation and function of immunocompetent cells. Despite the progress made in the last few years, a large number of questions still remain to be addressed and answered. We can only mention a few here. Are oxygenated sterols present in vivo in the lipoprotein fractions of the serum? If so, does the amount of such sterols fluctuate with changes in the diet? What are the effects of oxygenated sterols on the growth of spontaneous tumors? How do inhibitors of sterol synthesis affect the interaction of the immune system with tumor cells? By which mechanisms do inhibitors of sterol synthesis influence the primary and secondary immune response in mammals?

In mammalian cells, cholesterol is the most abundant single molecular species in the plasma membrane. It appears to have a unique structural function, namely, to modulate the so-called membrane fluidity. In order to understand the role of lipids and how they influence immunological phenomena as well as tumor growth, a thorough understanding of the role of cholesterol and its biosynthesis in mammalian cells will be an unavoidable necessity in the future.

References


Cholesterol and Its Biosynthesis in Normal and Malignant Lymphocytes

H. J. Heiniger


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/41/9_Part_2/3792

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/41/9_Part_2/3792.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.