SERUM LIPOPROTEINS AS CHOLESTEROL DONORS TO MYCOPLASMA MEMBRANES

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SUMMARY: *Mycoplasma hominis* and *Acholeplasma laidlawii* were grown in media in which a fraction of human serum lipoproteins provided the sole source of cholesterol. Increasing levels of very low density lipoproteins had an inhibitory effect on the growth of the organisms. Low and high density lipoproteins in all concentrations proved to be excellent sources of cholesterol. Both organisms were able to limit the amount of cholesterol taken up and to preferentially incorporate free cholesterol despite an excess of esterified cholesterol in the medium. When similar levels of free cholesterol were provided by low density or high density lipoproteins, the organisms incorporated from 20-45% more cholesterol from the former. This preference for cholesterol from low density lipoproteins partially supports the theory that the low density lipoproteins act as a donor while the high density lipoproteins are a scavenger of cholesterol.

Understanding the mechanism of cholesterol uptake by cells from the freely circulating plasma lipoproteins* has taken on new significance, as an increasing amount of evidence suggests that faulty control of cholesterol uptake may be a fundamental cause of atherosclerosis (1). We propose using mycoplasmas as a model system for investigating the role of serum lipoproteins as cholesterol donors. The rationale behind this is that these minute (less than 0.5 μm in diameter) parasitic procaryotes are capable of incorporating large

*Abbreviations: VLDL, very low density lipoproteins, d <1.006 g/ml; LDL, low density lipoproteins, d 1.006-1.063 g/ml; HDL, high density lipoproteins, d 1.063-1.21 g/ml.

quantities of cholesterol into their plasma membrane, and in fact most of them require cholesterol for growth. Unlike all other procaryotes the mycoplasmas have neither intracytoplasmic membranes nor cell walls, hence their plasma membrane is directly exposed to the growth medium and can be readily isolated by osmotic lysis of the cells (2). In addition, these organisms cannot synthesize or esterify cholesterol nor hydrolyze cholesteryl esters (3), and since they are incapable of pinocytosis, their uptake of cholesterol can be observed as a strictly membrane phenomenon (2).

Previous work from this laboratory, using the sterol-requiring *Mycoplasma* species and the closely related sterol-nonrequiring *Acholeplasma* species, has indicated that the organisms take up cholesterol through a physical adsorption process, independent of metabolic activity (4-6). Cholesterol was provided in these studies as part of a Tween 80-cholesterol complex or as part of a bovine serum fraction (Difco PPLO serum fraction). Since serum lipoproteins are the natural donors of cholesterol to the parasitic mycoplasmas, as well as to tissue cells, we decided to extend our studies by utilizing lipoproteins as the cholesterol source. This paper reports the results of these experiments and relates the findings to current concepts of the functioning of serum lipoproteins.

MATERIALS AND METHODS

Mycoplasma hominis and *Acholeplasma laidlawii* were grown in a modified Edward medium (7) in which the PPLO serum fraction was replaced by 0.5% fatty-acid-poor bovine serum albumin and elaidic acid (20 μg/ml). Cholesterol was added as a component of human VLDL, LDL, or HDL prepared according to Havel *et al.* (8). In some experiments LDL or HDL were labeled with ^{125}I as described by Bilheimer *et al.* (9), and more than 97% of the radioactivity was associated with the apoprotein moieties. *A. laidlawii* was harvested after 18 h and *M. hominis* after 36 h of incubation at 37 °C. Cell membranes were isolated by osmotic lysis (7) and extracted with chloroform:methanol (2:1, v/v). The lipid extracts from the membranes and from the lipoprotein fractions were analyzed for total, free (unesterified), and esterified cholesterol (3, 10) and for lipid phosphorus (11).

RESULTS

M. hominis and *A. laidlawii* incorporated free cholesterol from all three lipoprotein species. However, VLDL in concentrations required to provide free cholesterol concentrations exceeding 10 µg/ml medium inhibited growth of both organisms. This may have been due to the release of large quantities of free fatty acids by the action of mycoplasma lipases (12) on the VLDL triglycerides. Another possibility is that VLDL has a regulatory effect on a vital enzymatic activity. Very recently VLDL was found to stimulate the Mg^{++}-ATPase of the erythrocyte membrane (13). LDL and HDL enabled excellent growth of the mycoplasmas, as good or better than growth in medium supplemented with horse serum, the conventional cholesterol source in mycoplasma media.

Table I shows the effects of increasing concentrations of LDL and HDL on the cholesterol content of the mycoplasma membranes. *M. hominis* cells incorporated more cholesterol when larger quantities were available in the medium. However, LDL appeared to be a better donor of cholesterol to the membranes than HDL. At similar cholesterol concentrations 20-45% more cholesterol was taken up by cells grown on LDL when compared to cells grown on HDL. The ratio of free to esterified cholesterol in *M. hominis* membranes was found to increase with increasing free cholesterol content of the medium, despite the large excess of esterified cholesterol. The ratio of free to esterified cholesterol is much lower in HDL than in LDL, but the ratios found in the membranes of cells grown on HDL were higher than those from cells grown on LDL. Thus, the selectivitiy in the incorporation of free cholesterol over esterified was more pronounced with respect to HDL than to LDL. The uptake of cholesterol by the organisms was reflected by the increase in the ratio of free cholesterol to lipid phosphorus. In all cases the ratio was greater than that found in HDL, while it exceeded that found in LDL only at the two highest LDL concentrations.

TABLE I. Cholesterol uptake by *Mycoplasma hominis* and *Acholeplasma laidlawii* from serum lipoproteins

Lipoproteins added	Cholesterol in medium[A]			Cholesterol in membranes[B]				
				M. hominis			*A. laidlawii*	
	Free cholesterol (μg/ml)	Free/ esterified cholesterol (molar ratio)	Free cholesterol/ lipid Pi (molar ratio)	Cholesterol/ protein (μg/mg)	Free/ esterified cholesterol (molar ratio)	Free cholesterol/ lipid Pi (molar ratio)	Cholesterol[C]/ protein (μg/mg)	Cholesterol[C]/ lipid Pi (molar ratio)
LDL	7.2	0.33	1.11	66.2	1.09	0.83	25.7	0.26
LDL	28.2	0.33	1.11	78.4	1.49	0.92	50.3	0.55
LDL	72.0	0.33	1.11	107.4	2.50	1.16	45.9	0.65
LDL	144.0	0.33	1.11	111.1	3.58	1.33	52.3	0.55
HDL	6.0	0.25	0.13	49.4	1.68	0.59	35.4	0.44
HDL	24.0	0.25	0.13	55.1	3.06	0.81	39.8	0.53
HDL	60.0	0.25	0.13	62.1	3.50	0.88	31.3	0.44

A The isolated lipoproteins were added to the growth medium in quantities sufficient to provide the indicated levels of free cholesterol.

B Average levels of cholesterol (μg/mg membrane protein) in organisms grown on medium supplemented with horse serum were 72 (with ratio of free to esterified cholesterol of 2:1) and 31 (with no esterified cholesterol) for *M. hominis* and *A. laidlawii* respectively.

C Only free cholesterol was found to have been incorporated into the *A. laidlawii* membranes.

TABLE II. Comparison of binding of the protein and cholesterol moieties of LDL and HDL by mycoplasma membranes

^{125}I-labeled lipoportein[A]		*M. hominis*		*A. laidlawii*	
Type	Radioactivity in medium (total cpm)	Percent of lipoprotein ^{125}I protein bound	Percent of lipoprotein free cholesterol bound	Percent of lipoprotein ^{125}I protein bound	Percent of lipoprotein free cholesterol bound
LDL	3.72×10^6	0.78	10.5	0.70	5.8
LDL	7.40×10^6	0.99	8.3	0.52	3.4
HDL	1.04×10^7	0.07	20.0	0.02	8.8
HDL	2.02×10^7	0.05	11.0	0.02	4.6

[A] LDL (specific activity 1.60×10^6 cpm/mg protein) or HDL (specific activity 1.96×10^6 cpm/mg protein) was added to the growth medium to provide cholesterol concentrations of 28 and 55 µg cholesterol/ml medium from LDL and 13 and 25 µg cholesterol/ml medium from HDL. More than 97% of the added label was associated with the apoprotein moieties of the lipoproteins. Radioactivity of isolated cell membranes was measured with a Packard Autogamma Scintillation spectrometer. The amount of radioactivity found in the membrane lipids extracted with chloroform:methanol was negligible.

The growth of *A. laidlawii* was about the same in all concentrations of LDL or HDL as well as in their total absence, reflecting its known independence from cholesterol. Table I shows that despite the high concentration of free cholesterol in the medium, the cells incorporated a maximum of only about 50 µg cholesterol/mg membrane protein. Except at the lowest lipoprotein levels, it was found that the cells incorporated more cholesterol from LDL than from HDL. Although much more esterified than free cholesterol was present in the medium, only the latter was incorporated into the membranes of *A. laidlawii*. The ratio of cholesterol to phospholipid in the membranes was in all cases lower than that found in LDL but exceeded that found in HDL.

The binding of the protein moieties of ^{125}I-labeled LDL and HDL to mycoplasma membranes is compared to the uptake of cholesterol in Table II. The table shows that binding of ^{125}I-LDL protein exceeded that of ^{125}I-HDL protein by 10 to 30 fold. In addition, the table shows that a much higher percentage of cholesterol derived from the lipoprotein was taken up as compared to the protein moiety, indicating that the binding of the labeled protein was not directly related to that of cholesterol. Moreover, the labeled material bound to the membranes of *A. laidlawii* could not represent intact lipoprotein particles, as these particles contain large amounts of esterified cholesterol; and no esterified cholesterol was found associated with the *A. laidlawii* membranes.

DISCUSSION

Animal cells in culture have been employed as a model system for studying cholesterol uptake from lipoproteins (14, 15). However, the interpretation of the results obtained with tissue cells is complicated by the endogenous synthesis of sterols, the esterification of free cholesterol by the cells (15), and by the fact that lipoproteins are pinocytosed by tissue cells (16). Moreover, difficulties in separating the plasma membrane of the tissue cells from the intracytoplasmic membranes hamper the analysis of the direct interaction of the plasma membrane with the lipoprotein particle.

Results from experiments utilizing mycoplasma as a model system show that both *M. hominis* and *A. laidlawii* appear to possess effective mechanisms for restricting the amount of free or esterified cholesterol taken up from the growth medium. Models suggested for the structures of human LDL and HDL usually postulate the presence of free cholesterol near the surface of the particle where it may be readily exchanged (17, 18). Esterified cholesterol is usually thought to occur in a more

secluded region, perhaps near the center of the particle. The location of the esterified cholesterol may in itself restrict the amount which could be transfered from an intact particle to the cell membrane, or the esterified cholesterol may be as readily available as free cholesterol, while the ability of the membranes to incorporate large amounts of it may be restricted (19). It has been suggested that LDL may be a donor of cholesterol while HDL may be a scavenger of cholesterol (14, 15, 20). Our results showing the greater uptake of cholesterol from LDL than from HDL and the greater affinity of the cells to ^{125}I-protein from LDL lend support to this suggestion.

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