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The role of macrophage in lipid accumulation

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SUMMARY

Engorgement of macrophages with cholesterol is the defining pathological characteristic of atherosclerotic plaques, the cause of most heart attacks and strokes. Activated human macrophages uptake low density lipoprotein (LDL) by the bulk-phase fluid, and aggregated LDL by patocytosis. After incorporation of LDL and aggregated LDL into macrophages, neutral cholesterol esterase is decreased and cholesterol efflux is also deceased, resulting that macrophages become foam cells.

INTRODUCTION

Engorgement of macrophages with cholesterol is the defining pathological characteristic of atherosclerotic plaques, the cause of most heart attacks and strokes. Cholesterol-loaded macrophages secrete plaque disrupting matrix metalloproteinases and produce tissue factor that promotes thrombosis when plaques rupture. Thus, how macrophages accumulate cholesterol and become foam cells has been the subject of intense investigation.

Low density lipoprotein (LDL), the major carrier of blood cholesterol, has been implicated in the buildup of cholesterol in atherosclerotic plaques. Previously, native

LDL could not be shown to cause foam cell formation because the cellular receptor that binds LDL is poorly expressed on differentiated macrophages, and down regulates during cholesterol uptake limiting total cholesterol accumulation. Moreover, the LDL receptor is not expressed in human atherosclerotic plaques. However, although one popular hypothesis of foam cell formation involves LDL oxidation, human monocyte-derived macrophages do not become foam cells even if macrophages are incubated with oxidized LDL. Then, it has investigated how modification of macrophages rather than modification of LDL affects macrophage metabolism of the LDL. We show that macrophage foam cell formation can occur with native LDL when macrophages are activated with phorbol-12-myristrate-13-acetate (PMA), called " the bulk-phase fluid" (1).

Aggregation of LDL (AgLDL), which is one of modified LDL, may also contribute to its retention in developing atherosclerotic lesions, which is named as "patocytosis" (2). Furthermore, patocytosis induces not only foam cell formation but also release of cholesterol in the presence of plasminogen (3).

Furthermore, macrophages accumulate excessive cholesterol ester by altering macrophage biology. Although macrophages can release free cholesterol by metabolizing using acid cholesterol esterase, acyl CoA: cholesterol acyltransferase, and neutral cholesterol esterase, macrophages become foam cells by imbalance of uptake and release of cholesterol after engorgement of excessive LDL. In this study, it is introduced that human macrophages shows new slights of uptake of LDL (the bulk-phase fluid) and aggregated LDL (patocytosis), including changing intracellar cholesterol metabolism.

MATERIALS AND METHODS

Normal human monocytes purified with counter flow centrifugal elutriation were cultured for 2 weeks. Macrophage cell-association and degradation of ^{125}I-LDL were determined according to the methods of Goldstein et al (4). Unesterified and esterified cholesterol contents of macrophages and LDL were determined according to the fluorometric method of Gamble et al (5). Fluid-phase endocytosis was determined by incubating macrophages with ^{3}H-sucrose. The volumes of endocytosed fluid were calculated and expressed as μl/mg cell protein. The binding of ^{125}I-LDL to macrophages was performed according to previously described methods (3). Activity of cholesterol esterase was performed according to previously described methods (6).

RESULTS

1) PATOCYTOSIS

Patocytosis. Human monocyte-derived macrophages accumulate AgLDL by a unique endocytic pathway. In this actin-dependent process AgLDL induces a labyrinth of surface-connected compartments (SCC) and accumulates within them. This uptake pathway was maned as patocytosis, after the Latin word patere, meaning to lie open. The protein component of LDL, apoB, and other hydrophobic materials can stimulate patocytosis. After accumulating within macrophage SCC, some AgLDL is transported to lysosomes where it undergoes degradation followed by ACAT-dependent re-esterification of LDL cholesterol. However, most AgLDL remains undegraded within SCC.

Release of AgLDL from Macrophage SCC Was Mediated by Plasmin Derived from

Serum. After macrophages accumulated ^{125}I-AgLDL within SCC (for either 5 or 24 h) and were then exposed to LPDS for 24 h, macrophages released much of their accumulated ^{125}I-AgLDL back into the culture medium. Most ^{125}I-AgLDL was released as trichloroacetic acid-insoluble material indicating the presence of relatively intact LDL protein. This occurred for LDL aggregated by vortexing. Electron microscopy confirmed loss of AgLDL from macrophage SCC upon exposure of macrophages to LPDS. Plasmin is a serum serine protease that shows resistance to serum protease inhibitors when it is bound to the macrophage cell surface. Serum plasminogen is converted to active plasmin by urokinase and tissue plasminogen activators both of which are produced by macrophages within atherosclerotic lesions.

2) BULK FLUID-PHASE ENDOCYTOSIS

PMA stimulates macrophage uptake and degradation of LDL causing cholesterol accumulation. PMA-activated human monocyte-derived macrophages show a progressive time and concentration-dependent increase in macrophage cholesterol content during incubation with native LDL at levels similar to the LDL concentration in artery intima. This results in macrophages loaded with lipid droplets similar in appearance to foam cells isolated from atherosclerotic lesions. In contrast to macrophages incubated with LDL plus PMA, macrophages incubated with LDL without PMA show only a slight increase in cholesterol content.

PMA-stimulated increase in macrophage cholesterol content is accompanied by uptake and degradation of LDL. The net total ^{125}I-LDL uptake stimulated by PMA (i.e., cell-associated + degraded ^{125}I-LDL with PMA minus cell-associated + degraded ^{125}I-

LDL without PMA) is linear with increasing ^{125}I-LDL concentration. On the other hand, the total ^{125}I-LDL uptake without PMA shows saturation with increasing ^{125}I-LDL concentration. At 500 μg/ml ^{125}I-LDL, the total macrophage uptake of ^{125}I-LDL is between 5-6 times greater in the presence of PMA compared with the absence of PMA. In both cases, macrophages degrade the majority of ^{125}I-LDL taken up. Experiments show that the total amount of ^{125}I-LDL uptake can account for cholesterol accumulated by PMA-stimulated macrophages incubated with ^{125}I-LDL. Thus, whole LDL particle uptake rather than selective cholesteryl ester uptake occurs. PMA-stimulated uptake of LDL leads to ACAT-dependent cholesterol esterification. This is consistent with the known action of ACAT to re-esterify lipoprotein-derived unesterified cholesterol after lysosomal hydrolysis of lipoprotein-derived cholesteryl ester (acid cholesterol esterase). Furthermore, neutral cholesterol esterase decreased after cholesterol accumulation by the uptake of LDL in presence of PMA.

The LDL receptor does not mediate PMA-stimulated uptake of LDL. Reductive methylation of LDL apoB blocks its binding to the LDL receptor. This LDL modification decreases PMA-stimulated macrophage cholesterol accumulation by only a small amount, less than 20%. However, the LDL receptor is not responsible for the small decrease in cholesterol accumulation that occurrs with reductively methylated LDL. The anti-LDL receptor blocking monoclonal antibody, C7, added at a 10-fold molar excess based on protein shows no inhibition of PMA-stimulated cholesterol accumulation as compared to an isotype-matched control antibody. Moreover, we found that PMA treatment of macrophages does not increase ^{125}I-LDL binding.

Role of fluid-phase (i.e., bulk-phase) endocytosis in macrophage LDL uptake. Because PMA does not stimulate increased binding of LDL to macrophages and PMA is known to stimulate fluid-phase endocytosis in macrophages, it was examined whether fluid-phase endocytosis could explain the PMA-stimulated uptake of LDL. PMA treatment of macrophages increased macrophage uptake of medium during a 24-hour incubation from 5.8 ± 0.4 to 25.5 ± 1.2 μl/mg cell protein. Comparison of the amount of ^{125}I-LDL uptake that occurred with the amount of ^{125}I-LDL uptake predicted from fluid-phase endocytosis of medium shows that an average of 83 ± 4% of ^{125}I-LDL uptake can be accounted for from fluid-phase endocytosis

CONCLUSION

Macrophages uptake native LDL by bulk-phase fluid and aggregated LDL by patocytosis, and then become foam cell. After uptake of LDL particles, macrophages modified cholesterol metabolism, resulting reduce cholesterol efflux accompanied by decrease of neutral cholesterol esterase activity. However, aggregated LDL was released from SCC by serum plasminogen. Although macrophages may works as protector against excessive LDL of hypercholesterolemia by bulk-phase fluid and patocytosis, macrophage activation could influence atherosclerotic plaque cholesterol accumulation and progression.

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