Multinucleated Variant Endothelial Cells (MVECs) Have a Greater Capacity for LDL Cholesterol Uptake Than Typical Mononuclear Endothelial Cells (TECs)

Osamu Tokunaga, Toshimi Satoh, and Shi Yu

Department of Pathology, Saga Medical School, Nabeshima, Japan.

The existence of large endothelial cells in the human aorta, especially on atherosclerotic lesions has been reported. They have multiple nuclei and are called "multinucleated variant endothelial cells (MVECs)". In the present study caveolin expression was demonstrated in both MVECs and small typical endothelial cells (TECs). Caveolin was expressed diffusely as fine particles, and caveoles were expressed as prominent accumulations of caveolin in the cytoplasm. LDL was bound to the endothelial surface. With double immunostaining for caveolin and LDL, the location of LDL corresponded to the immunoreactive caveoles. Over time, large dots of LDL appeared in MVECs, whereas a few fine particles remained in TECs. An electron microscopic chase study of LDL-gold uptake identified many LDL-gold particles in plasmalemmal vesicles and in endosomes or lysosomes of MVECs, but only a few particles were found in TECs. Gold containing vesicles often were located near the abluminal surface. The number of LDL-gold particles was 4.5 times greater per unit area in MVECs than in TECs. Some of the gold particles were located in the subendothelial collagen matrix. These findings indicate that MVECs have a greater capacity of LDL cholesterol uptake followed by transport to the subendothelial matrices than TECs, and that MVECs contribute to the development and advancement of atherosclerotic lesions. *J Atheroscler Thromb*, 2002 ; 9 : 35-41.

Key words : MVEC, TEC, Caveolin, LDL-gold, LDL uptake

Introduction

Lipid accumulation in the aortic intima is an early event of atherogenesis, and excess lipids are taken up by macrophages and smooth muscle cells, which migrate to the intima from the media. Lipids are integral to the advancement and complications of atherosclerosis. In large arteries, plasma LDL and other lipoproteins are taken up by endothelial cells and either used by these endothelial cells or transported to the intima where atherosclerosis begins. LDL is trapped in the matrices of the intima and may be oxidized or modified, thus becoming

chemotactic for blood monocytes. Once the monocytes differentiate to macrophages, phagocytic activity begins on the modified LDL through the scavenger receptors, and the macrophages transform into foam cells characterized by a massive accumulation of cholesterol and cholesteryl esters in lysosomes and cytoplasmic lipid droplets (1-3). Smooth muscle cells in the intima are stimulated to proliferate and secrete extracellular matrices by platelet-derived growth factor, basic fibroblast growth factor, and transforming growth factor- β (4). These factors further enhance the expression of LDL receptors (5-7) and scavenger receptors (8-10) and stimulate LDL uptake.

Increasing interest has been paid to the role of caveolae in cellular cholesterol transport (11-13). These fine invaginations of the cell membrane originally were described in endothelial cells, where they participate in macromolecular transport by transcytosis (14). Later, they were implicated in a variety of functions, many

Address for correspondence: Osamu Tokunaga, MD, PhD, Department of Pathology, Saga Medical School, Nabeshima, Saga 849-8501, Japan.

E-mail: tokunao@post.saga-med.ac.jp

Received August 2, 2001.

Accepted for publication August 20, 2001.

related to signal transduction (15). Caveolin is an integral membrane protein, which was first identified as a major substrate for v-Src in Rous sarcoma virus-transformed chick embryo fibroblasts. Now termed Caveolin-1, it has been localized to non-clathrin-coated invaginations of the plasma membrane called caveolae. Caveolin-1 occurs in two forms, α and β , due to alternate splicing of the mRNA. Caveolin-1 forms large lipid-binding homo-oligomers that are believed to play a role in caveolae formation. It also functions as a scaffolding protein that concentrates and organizes signaling molecules, a purported role supported by the fact that caveolin-1 interacts directly with inactive Ras and G-protein α subunits (16–18). In addition, caveolin has been identified as a component of vesicles derived from the trans-Golgi network (19), and caveolin cycles between plasma membrane caveolae and the Golgi complex (20). Caveolin mediates the transport of free cholesterol to the plasma membrane and the subsequent efflux from the cell (21, 22) and is up-regulated by free cholesterol (23).

Large multinucleated endothelial cells are often found in the human aorta and are called multinucleated variant endothelial cells (MVECs). The number of MVECs increases with atherosclerosis grade and aging (24). Preliminary investigations of MVECs revealed over-expression of the LDL receptor on the surface of MVECs (25, 26) and enhanced uptake of native LDL (27). The aim of this study was to determine the degree of MVEC participation in the cholesterol metabolism of the vascular wall, how the caveoles behave in uptake and transport of LDL in MVECs, and the relationship of MVECs to the development of atherosclerosis.

Materials and Methods

Isolation and culture of human aortic endothelial cells

Endothelial cells were harvested and cultured from aortae at autopsy according to a modified version of the previously reported method (25). The cells were cultured in an autoclavable RPMI 1640 (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with L-glutamine (0.294 g/l; GIBCO, Grand Island, NY), 20% fetal bovine serum (FBS; Microbiological, Associates, Logan, UT), epidermal growth factor (5 μ g/ml, Wako Pure Chemical, Osaka, Japan), hydrocortisone (1 μ g/ml, Becton Dickinson Labware, Bedford, MA), 15 μ g/ml endothelial-cell growth supplement (ECGS; Collaborative Research, Lexington, KY), and 100 μ g/ml of heparin. An antibiotic cocktail consisting of 15 μ g/ml of gentamicin, 1.5 μ g/ml of amphotericin B, 50 μ g/ml of ampicillin, and 2 μ g/ml of minomycin was added to the medium for primary culture. After 2 days, the old medium was removed by suction, and the culture was replenished with fresh medium containing only gentamicin.

On the occasion of aorta preparation at autopsy, the aortas were treated with 0.3% silver nitrate. Then a layer

of endothelial cell lining was peeled off from the sub-endothelial intima by an en-face Hutchen preparation method under a stereomicroscope (SZH10, Olympus, Tokyo), mounted on a slide glass, and stained with hematoxylin. The endothelial cell linings were transferred surface down onto resin-coated slide glasses by touch preparation and stained with Oil Red O.

Double immunofluorescence

For immunohistologic staining, the endothelial cells were cultured on a cover glass and briefly fixed in either buffered 10% formalin or cold acetone. After being washed with phosphate-buffered saline (PBS, pH 7.4), the cells were immunoreacted with primary/secondary antibody combinations as follows; anti-human caveolin antibody (Clone C060, IgM, Transd La, $\times 500$)/rhodamine-conjugated anti-mouse IgM polyclonal antibody ($\times 100$), anti-human apolipoprotein B antibody (Clone NP, Boehringer Mannheim, $\times 200$)/FITC-conjugated anti-mouse IgG polyclonal antibody ($\times 100$), and anti-human LDL receptor peptide (LDL-R, Ab-1, Oncogene Science, Uniondale, NY, $\times 200$)/FITC-conjugated anti-mouse IgG polyclonal antibody ($\times 100$). Nuclei were counterstained with 0.1 μ g/ml 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) in the antifade p-phenylene-diamine dihydrochloride.

LDL-gold conjugate

A total of 50 ml of 0.01% gold solution (5 nm, British BioCell, Cardiff, UK) was activated by raising the pH to 9.0 with 100 nM NaOH. Then 2.5 ml of LDL solution, diluted to a concentration of 0.1 μ g/ml with 0.15 M NaCl (pH adjusted to 9.2), was added dropwise to the gold solution during vigorous stirring. After 5 min, 5 ml of the filtered 10% bovine serum albumin (BSA) at pH 9.0 was added to the solution, and stirring continued for 10 min. The gold-conjugate was spun down at $45,000 \times g$ for 1 h at 4°C, and the precipitate was suspended in 2 ml of buffer A containing Tris-HCl (pH 8.2), 1% BSA, and 0.1% sodium azide. The suspended gold-conjugate was overlaid on 10 ml of 10%–30% glycerol gradient in buffer A and centrifuged at $125,000 \times g$ in a swing rotor at 4°C. The resulting gradient was fractionated every 2 ml, and each fraction was examined by electron microscopy (EM). The most proper fractions were combined and the protein concentration was adjusted to that of the original LDL.

LDL-gold uptake assay

For LDL-gold uptake assay, human aortic endothelial cells were cultured for 48 h in RPMI1640 with LDL-deficient serum on a 3% type-I collagen gel (Cellmatrix, Nitta Gelatin, Osaka, Japan) in transwells, that were 12 mm in diameter and 0.4 μ m in pore size (Corning Costar, Cambridge, MA). The endothelial cells were then incubated for 1 h at 37°C with the same fresh medium containing 200 μ g/ml of LDL-gold conjugate, followed by incubation with

Fig. 1 En-face silver nitrate treatment of the human atherosclerotic plaque. The nonatherosclerotic flat area is generally covered by typical small endothelial cells (A). $\times 200$. Most of the elevated lesion is covered by giant cells of various sizes and shapes (B). $\times 200$. These giant cells with 2-10 or more nuclei are called multinucleated variant endothelial cells (MVECs) (C). $\times 600$. Oil Red O staining on touch preparation from the aortic endothelial lining. Lipid droplets are seen mostly in the MVECs (D, arrow). $\times 400$.

Fig. 2 Double immunostaining of caveolin (A, red) and LDL (B, green). Caveolin is stained as diffuse fine particles. Large immunoreactive LDL corresponds to large, dense areas of immunoreactive caveolin (arrow) in MVEC, whereas few fine particles were noted in TECs. $\times 600$.

medium containing 20% FBS. During this incubation, samples were collected at 5 min, 1 h, 3 h, 6 h, and 12 h, and fixed with 4% glutaraldehyde. They were processed according to a conventional method for electron microscopic observation and examined in a JEM-1220 transmission electron microscope (JEOL, Tokyo, Japan) at 85 kV.

Image analysis of immunoreactive caveolin

The total cell area and the intensity of immunofluorescence of TEC and MVEC were analyzed by LP Lab

Scientific Imaging Software for Macintosh (Scanalytics, Inc., Fairfax, VA, and MacScope, Mitani Corp., Fukui, Japan). The theoretical principle of this software is based on RGB color density and 8-bit greyscale images.

Results

In vivo presence of MVECs and lipid droplets

The border of the endothelial cells was outlined well by silver nitrate treatment. The flat areas were covered with small typical endothelial cells (Fig. 1A). Atherosclerotic

lesions were often covered by large polygonal endothelial cells. Their size and shape varied, and giant endothelial cells often were observed. These giant cells were up to ten times larger than the surrounding small endothelial cells (Fig. 1B), and they were multinucleated (Fig. 1C). Double nuclei or triple nuclei were also seen. Thus, a heterogeneous population of endothelial cells was evident on atherosclerotic lesions.

Touch preparation of the aorta was used to transfer the endothelial lining to the slide glass and demonstrated the *in vivo* presence of MVECs. Oil Red O staining revealed many lipid droplets in the MVECs (Fig. 1D).

Double immunocytochemistry with caveolin and LDL

Caveolin was stained as diffuse fine particles on the

Table 1 Image analysis of cell area and caveolin density between MVEC and TEC

Cell Number	Average area /cell ($\times 10 \mu^2$)	Average caveolin density (unit/ μ^2)	Density deviation /cell (unit)
MVEC 100	$7.7 \pm 2.0^*$	71.5 ± 23.8	$35.1 \pm 6.4^*$
TEC 300	1.7 ± 1.2	71.8 ± 22.9	27.5 ± 10.1

* MVEC is significant against TEC.

Student *t* test, $p < 0.05$

cell surface (Fig. 2A). Caveolin and caveoles were distributed evenly in cytoplasm with slight perinuclear predisposition. The distribution pattern and density were almost the same in MVECs and small typical endothelial cells (TECs). LDL is supposed to react with its receptor on the cell surface, and this process was visible by FITC-labeled antibodies (Fig. 2B). Double immunocytochemistry revealed that LDL corresponded to large, dense areas of immunoreactive caveolin (Fig. 2A and B, arrow), whereas few fine particles were noted in TECs.

Image analysis of immunoreactive caveolin

TEC and MVEC cell areas were traced and combined for analysis. The average positive area of caveolin per MVEC was approximately 5 times greater than that of TEC, $7.7 \pm 2.0 \times 10 \mu^2$ vs $1.7 \pm 1.2 \times 10 \mu^2$ (Table 1 and Fig. 3A). The average caveolin density per μ^2 was the same as that of TEC, 71.5 ± 23.8 vs 71.8 ± 22.9 (Fig. 3B). This means that the density of the caveolin per unit area was the same between MVECs and TECs. However, the density deviation per cell was significantly greater in MVEC than that in TEC, 35.1 ± 6.4 unit vs 27.5 ± 10.1 unit ($p = 0.0016$) (Fig. 3C). This may reflect the presence of larger, dense droplets that were positive for caveolin, and were much larger than those of accumulation observed in most of the TECs. With double immunostainings for

Fig. 3 Image analysis of immunoreactive caveolin. MVEC has a 5-fold greater positive area than TEC. * $p < 0.01$ (A), but the average positive area per unit area is equivalent in both (B). Density deviation per cell is significantly greater in MVECs than in TECs (C). ** $p < 0.05$

Fig. 4 LDL-gold electron microscopy. LDL-gold is bound to the cell surface by 5 min (A). Then the particles are internalized (B) and present in plasmalemmal vesicles (C). $\times 100,000$.

Fig. 5 LDL-gold cumulative graph. The relative number of LDL-gold particles in cytoplasm of TECs peaks at 3 h of incubation (A). The actual number of gold particles is 4.5-fold greater per MVEC than that per TEC (B) when compared at 3 h of incubation. * $p < 0.01$ between MVEC and TEC cytoplasm.

caveolin and LDL, these immunoreactive caveolin droplets corresponded precisely to the LDL positive sites (Fig. 2B).

LDL-gold uptake by MVECs

After 5 min of incubation, LDL-gold particles were bound to the cell surface and in the indented-coated regions (caveoles) (Fig. 4A). After incubation at 37°C, LDL-gold became internalized (Fig. 4B) and was located primarily in endosomes, lysosomes, and plasmalemmal vesicles (Fig. 4C).

The number of gold particles was counted each at cell surface, cytoplasm or vesicle, and subendothelial matrix by electron microscopy. In TECs the number of gold particles in the cytoplasm and vesicles increased with time, peaked at 3 h, and then decreased (Fig. 5A). Therefore, the comparison of LDL-gold uptake between MVECs and TECs was made at 3 h of incubation (Fig. 5B).

The number of LDL-gold particles per unit area was greater in MVECs than in TECs. An approximate 4.5-fold increase of LDL-gold uptake was noted in MVECs. This difference was also demonstrated by electron microscopy. In MVEC many foci of gold particles were found (Fig. 6A, arrows), but there were few or only one focus in TEC (Fig. 6B, arrow). LDL-gold particles were present in plasmalemmal vesicles and in endosomes or lysosomes. Most LDL-gold particles were located at the lower part of the cell, with some existing in an aggregated form (Fig. 6A, inset). Sometimes they opened on the abluminal front of the endothelial cells. At the end of incubation period, many particles were transported into and scattered within the subendothelial collagen matrix.

Discussion

The relation of MVECs, or giant endothelial cells, to atherosclerosis has been reported (24). MVECs have been formed experimentally in rabbits fed a hypercholesterol diet (28). However, the pathogenesis and roles of MVECs remain partially understood.

The function of MVECs in the human aorta remains unknown. The development of anti-LDL-receptor antibody has made it possible to investigate the presence of surface membrane receptors (1). When we cultured endothelial cells in serum deficient medium and starved them of LDL cholesterol, the LDL receptor was expressed at the maximal level on the cell surface membrane. Under this condition, MVECs strongly expressed LDL receptors, which were subsequently internalized. TECs, however expressed few receptors on the cell surface. Gold-labeled LDL was visible under an electron microscope (29). Human fibroblasts and umbilical vein endothelial cells were bound to LDL-gold on the surface membrane when they were cultured in a medium with LDL deficient serum (30).

Caveolin-1 is localized to nonclathrin-coated invaginations of the plasma membrane, called caveolae, and functions as a signaling molecule. In addition, caveolin has been identified as a component of vesicles in the trans-Golgi network, and it cycles between the plasma membrane caveolae and the Golgi complex (15). Caveolin immunocytochemistry and tracking of ultrastructural uptake of LDL-gold over 5 to 60 min revealed that LDL immediately bound to the MVECs surface and is taken up in the scaffold of the caveolae (Fig. 4A-C). Additionally, LDL is transported to subendothelial matrices. These findings are consistent with those of cholesterol transport in arterial smooth muscle cells (31). Excess cholesterol is discharged from caveolin/caveolae on the cell surface (21, 22). MVECs proved to have a 4.5-fold greater capacity for LDL-gold uptake per cell than TECs (Fig. 5). The elevated LDL-gold uptake was expected from a previous immunocytochemical study of the LDL receptor (25). Image analysis of the immunor-

Fig. 6 Many foci of gold particles were found in MVEC (A, arrows), but only one focus is seen in TEC (B, arrow). $\times 2,500$. Many particles exist in endosomes and lysosomes. Inset: Representative area of gold particles shown in square in A and B, respectively. $\times 50,000$.

ective caveolin showed that the density deviation per cell was significantly greater in MVECs than in TECs although the caveolin distribution per unit area was equivalent. This reflects the larger LDL droplets (Fig. 2) and gold-containing vesicles (Fig. 6) in MVECs compared with TECs. LDL-gold internalization may be disturbed in MVECs, and excess cholesterol may be released to the subendothelial matrix. Thus, a much greater amount of LDL is transported from the circulating blood to subendothelial space through MVECs than through TECs. MVECs would be the greater supplier of LDL to the vascular wall at atherosclerotic lesions. MVECs provide LDL cholesterol, which is an essential component for fatty streak and atheroma, to the vascular wall, and actively participate in the development and advancement of atherosclerosis.

References

- (1) Brown MS and JL Goldstein: Lipoprotein receptors in the liver. *J Clin Invest*, 72: 743-747, 1983
- (2) Shio H, Haley NJ, and Fowlers S: Characterization of lipid-laden aortic cells from cholesterol-fed rabbits. III. Intracellular localization of cholesterol and cholesteryl ester. *Lab Invest*, 41: 160-167, 1979
- (3) Fowler S, Shio H, and Haley NJ: Characterization of lipid-laden aortic cells from cholesterol-fed rabbits: IV Investigation of macrophage-like properties of aortic cell populations. *Lab Invest*, 41: 372-378, 1979
- (4) Ross R and Fuster V: The pathogenesis of atherosclerosis. In: *Atherosclerosis and coronary artery disease*. ed by Fuster V, Ross R, and Topol EJ, pp. 441-460, Lippincott-Raven Publishers, Philadelphia, 1996
- (5) Chait A, Ross R, Albers JJ, and Bierman EL: Platelet-derived growth factor stimulates activity of low density lipoprotein receptors. *Proc Natl Acad Sci USA*, 77: 4084-4088, 1980
- (6) Nicholson AC and Hajjar DP: Transforming growth factor- β up-regulates low density lipoprotein receptor-mediated cholesterol metabolism in vascular smooth muscle cells. *J Biol Chem*, 267: 25982-25987, 1992
- (7) Roth M, Emmons LR, Perruchoud A, and Block LH: Expression of the low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase genes are stimulated by recombinant platelet-derived growth factor isomers. *Proc Natl Acad Sci USA*, 88: 1888-1892, 1991
- (8) Gong Q and Pitas RE: Synergistic effects of growth factors on the regulation of smooth muscle cells scavenger receptor activity. *J Biol Chem*, 270: 21672-21678, 1995
- (9) Inaba T, Gotoda T, Shimano H, Shimada M, Harada K, Kozaki K, Watanabe Y, Hoh E, Motoyoshi K, Yazaki Y,

- and Yamada N: Platelet-derived growth factor induces c-fms and scavenger receptor genes in vascular smooth muscle cells. *J Biol Chem*, 267 : 13107-13112, 1992
- (10) Li HM, Freeman MW, and Libby P: Regulation of smooth muscle cell scavenger receptor expression *in vivo* by atherogenic diets and *in vitro* by cytokines. *J Clin Invest*, 95 : 122-133, 1995
 - (11) Fielding PE and Fielding CJ: Intracellular transport of low density lipoprotein derived free cholesterol begins at clathrin-coated pits and terminates at cell surface caveolae. *Biochemistry*, 35 : 14932-14938, 1996
 - (12) Ikonen E: Molecular mechanisms of intracellular cholesterol transport. *Curr Opin Lipidol*, 8 : 60-64, 1997
 - (13) Lamaze C and Schmid SL: The emergence of clathrin-independent pinocytic pathways. *Curr Opin Cell Biol*, 7 : 573-580, 1995
 - (14) Simionescu M and Simionescu N: Endothelial transport of macromolecules: Transcytosis and endocytosis. A look from cell biology. *Cell Biol Rev*, 25 : 5-78, 1991
 - (15) Couet J, Li S, Okamoto T, Scherer PE, and Lisanti MP: Molecular and cellular biology of caveolae. Paradoxes and plasticities. *Trends Cardiovasc Med*, 7 : 1103-1110, 1997
 - (16) Conrad PA, Smart EJ, Ying Y-S, Anderson RGW, and Bloom GS: Caveolin cycles between plasma membrane caveolae and the golgi complex by microtubule-dependent and microtubule-independent steps. *J Cell Biol*, 131 : 1421-1433, 1995
 - (17) Li S, Song GS, and Lisanti MP: Expression and characterization of recombinant caveolin. Purification by polyhistidine tagging and cholesterol-dependent incorporation into defined lipid membranes. *J Biol Chem*, 271 : 1199-1215, 1996
 - (18) Garcia-Cardena G, Fan R, Stern DF, Liu J, and Sessa WC: Endothelial Nitric Oxide synthase is regulated by tyrosine phosphorylation and interacts with caveolin-1. *J Biol Chem*, 271 : 27237-27240, 1996
 - (19) Kurzchalia TV, Dupree P, Parton RG, Kellner R, Virta H, Lehnert M, and Simons K: VIP21, a 21-kD membrane protein, is an integral component of trans-golgi-network-derived transport vesicles. *J Cell Biol*, 118 : 1003-1014, 1992
 - (20) Smart EJ, Ying Y-S, Conrad PA, and Anderson RGW: Caveolin moves from caveolae to the golgi apparatus in response to cholesterol oxidation. *J Cell Biol*, 127 : 1185-1197, 1994
 - (21) Fielding PE and Fielding CJ: Plasma membrane caveolae mediate the efflux of cellular free cholesterol. *Biochemistry*, 34 : 14288-14292, 1995
 - (22) Smart EJ, Ying Y-S, Donzell WC, and Anderson RGW: A role for caveolin in transport of cholesterol from endoplasmic reticulum to plasma membrane. *J Biol Chem*, 271 : 29427-29435, 1996
 - (23) Fielding CJ, Bist A, and Fielding PE: Caveolin mRNA levels are upregulated by free cholesterol. *Proc Natl Acad Sci USA*, 94 : 3753-3758, 1997
 - (24) Tokunaga O, Fan J, and Watanabe T: Atherosclerosis and age-related multinucleated variant endothelial cells. *Am J Pathol*, 135 : 967-976, 1989
 - (25) Satoh T, Sasatomi E, Yamasaki F, Ishida H, Wu L, and Tokunaga O: Multinucleated variant endothelial cells of human aorta: expression of tumor suppressor gene p53 and relationship to atherosclerosis and aging. *Endothelium*, 6 : 123-132, 1998
 - (26) Tokunaga O, Satoh T, Yamasaki F, and Wu L: Multinucleated variant endothelial cells (MVECs) in human aorta: Chromosomal aneuploidy and elevated uptake of LDL. *Semin Thromb and Hemost*, 24 : 279-284, 1998
 - (27) Wu L, Satoh T, and Tokunaga O: Formation of multinucleated variant endothelial cells *in vitro* and investigation of MVEC's features. *Fukuoka Acta Medica*, 90 : 377-391, 1999
 - (28) Tashiro K, Shimokama T, Haraoka S, Tokunaga O, and Watanabe T: Endothelial cell heterogeneity in experimentally-induced rabbit atherosclerosis. Demonstration of multinucleated giant endothelial cells by scanning electron microscopy and cell culture. *Virchows Arch*, 425 : 521-529, 1994
 - (29) Beesley J: Colloidal Gold. A new perspective for cytochemical marking. Royal Microscopical Society Handbook No.17, Oxford Science Publication. Oxford University Press, Oxford, 1989
 - (30) Haveks L, Mommaas-Kienhuis AM, Schouten D, Wit ED, Scheffer M, and Van Hinsbergh VWM: High-affinity uptake and degradation of acetylated low density lipoprotein by confluent human vascular endothelial cells. *Atherosclerosis*, 56 : 81-92, 1985
 - (31) Thyberg J, Calara F, Dimayuga P, Nilsson J, and Rengstrom J: Role of caveolae in cholesterol transport in arterial smooth muscle cells exposed to lipoproteins *in vitro* and *in vivo*. *Lab Invest*, 78 : 825-837, 1998