Contribution of Intimal Smooth Muscle Cells to Cholesterol Accumulation and Macrophage-Like Cells in Human Atherosclerosis

Sima Allahverdian, MD, PhD; Ali Cyrus Chehrouri; Bruce M. McManus, MD, PhD; Thomas Abraham, PhD; Gordon A. Francis, MD

Background—Intimal smooth muscle cells (SMCs) contribute to the foam cell population in arterial plaque, and express lower levels of the cholesterol exporter ATP-binding cassette transporter A1 (ABCA1) in comparison with medial arterial SMCs. The relative contribution of SMCs to the total foam cell population and their expression of ABCA1 in comparison with intimal monocyte-derived macrophages, however, are unknown. Although the expression of macrophage markers by SMCs following lipid loading has been described, the relevance of this phenotypic switch by SMCs in human coronary atherosclerosis has not been determined.

Methods and Results—Human coronary artery sections from hearts explanted at the time of transplantation were processed to clearly delineate intracellular and extracellular lipids and allow costaining for cell-specific markers. Costaining for oil red O and the SMC-specific marker SM α-actin revealed that 40±6% (n=15) of oil red O-positive foam cells were SMC derived. ABCA1 expression by intimal SMCs was significantly reduced between early and advanced atherosclerotic lesions, with no loss in ABCA1 expression by myeloid lineage cells. Costaining with the macrophage marker CD68 and SM α-actin revealed that 40±6% (n=15) of CD68-positive cells originated as SMCs in advanced human coronary atherosclerosis.

Conclusions—These findings suggest SMCs contain a much larger burden of the excess cholesterol in human coronary atherosclerosis than previously known, in part, because of their relative inability to release excess cholesterol via ABCA1 in comparison with myeloid lineage cells. Our results also indicate that many cells identified as monocyte-derived macrophages in human atherosclerosis are in fact SMC derived. (Circulation. 2014;129:1551-1559.)

Key Words: atherosclerosis ■ cholesterol ■ foam cells ■ macrophages ■ myocytes, smooth muscle

Smooth muscle cells (SMCs) are the main cell type in early arterial intimal thickenings and a major component of most stages of human atherosclerosis.1,2 Like monocyte-derived macrophages, SMCs express scavenger receptors and become foam cells on exposure to lipoproteins.3–5 Despite these findings, and the many descriptions of SMC-derived foam cells in human atherosclerotic lesions,6,7 the relative contribution of SMCs to total foam cell formation and macrophage-like cells in human atherosclerosis has not been studied. The apparent scarceness of intimal SMCs in mouse models of atherosclerosis5,9 has contributed to the deemphasis of these cells as contributors to foam cell formation, although a recent study suggests that SMCs lacking classic SMC markers are prevalent in mouse arterial intima.9 In comparison with monocyte-derived macrophages, there is a striking lack of literature regarding the mechanisms that control cholesterol metabolism in arterial SMCs.

In addition to cholesterol uptake, foam cell formation depends on the cell’s ability to release excess cholesterol, the rate-limiting component of which is mediated by the membrane lipid transporter ATP-binding cassette transporter A1 (ABCA1).11 We previously reported that ABCA1 mRNA and SMC-specific ABCA1 protein levels, as well, are diminished in the intimal layer of human coronary arteries.12 This suggested formation of SMC foam cells is due in part to their reduced ability to efflux excess cholesterol via ABCA1.12 To determine the relative importance of SMC cholesterol accumulation in the intima, in the current studies we also compared intimal SMC- and myeloid cell–specific expression of ABCA1 in the early and late stages of human atherosclerosis.

Coronary Heart Disease

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Previous studies have indicated that lipid loading induces the expression of macrophage markers and a gain of phagocytic activity by arterial SMCs. The presence of cells expressing both SMC and macrophage markers has been demonstrated in en face preparations of human aortic intima. These findings raise the possibility that some atherosclerotic lesion cells expressing macrophage markers, and assumed to be of monocyte origin, may be in fact be SMCs that have an altered phenotype following lipid loading. The extent of this phenotypic switch has not been previously investigated in human coronary atherosclerosis.

Our results suggest that, in human coronary intima, a very large proportion of foam cells are SMC rather than myeloid cell derived, the intimal SMCs in advanced lesions have a selective reduction in ABCA1 expression in comparison with myeloid-origin cells, and a large percentage of cells expressing macrophage markers in advanced atherosclerosis are derived from SMCs rather than monocytes.

Methods

Tissue Sections

Explanted hearts donated by consenting patients undergoing heart transplantation were obtained from the Cardiovascular Registry at the Centre for Heart Lung Innovation, University of British Columbia following approval by the University of British Columbia Clinical Research Ethics Board. Coronary artery tissues from 31 hearts containing sufficient foam cells for cell identification studies (Table I in the online-only Data Supplement). Coronary artery sections from 24 patients across the range of atherosclerosis stages were used for immunohistochemical studies (Table II in the online-only Data Supplement).

Lipid Fixation

Formalin-fixed human coronary artery tissues were processed to carefully preserve lipid content in paraffin sections. In brief, formalin-fixed tissues (1–2 mm thick) were kept in an emulsion of linoleic acid (60% practical grade, MP Biomedicals) and lecithin (Jamieson Natural Sources) in 70% ethylene glycol (Sigma) at 56°C for 3 to 5 days. Tissues were rinsed for at least 8 hours in several changes of 70% ethanol followed by several changes of distilled water, immersed in 2% chromic acid for 24 hours at 4°C, then rinsed in several changes of distilled water for 24 hours. Finally, tissues were placed in a 5% sodium bicarbonate solution for 24 hours followed by rinsing in water for at least 8 hours. Tissues were then embedded and cut in paraffin sections and stained with oil red O. This method preserves neutral lipids in tissues for subsequent lipid staining. Comparison of tissues fixed in this way and by frozen section showed a similar extent, intensity, and distribution of lipid staining in types I to IV human coronary atherosclerosis by the use of the 2 methods. Optimal cutting temperature–embedded frozen sections were analyzed for comparison as previously described.

Immunostaining

To determine the cell type of origin of foam cells, formalin-fixed, paraffin-embedded tissues with lipids preserved were cut in 5-μm-thick sections and stained histochemically with oil red O and counterstained with hematoxylin and eosin to ensure the presence of lipids in coronary artery sections. Once the presence of foam cells or extracellular lipid were confirmed in intimas, serial sections were double-stained with SM α-actin (Abcam) or CD45 (Dako) antibodies and oil red O. In brief, sections were dewaxed, rehydrated, and incubated in Tris/ethylenediaminetetraacetic acid pH 9.0 buffer for 30 minutes for antigen retrieval. Slides were then blocked with Dako universal block for 30 minutes followed by incubation with SM α-actin or CD45 antibodies overnight. AlexaFluor 488–conjugated donkey anti-mouse immunoglobulin G was used to detect SM α-actin and CD45.

Sections were then processed for oil red O staining with subsequent staining of nuclei using Hoechst 33342.

To determine the origin of cells expressing ABCA1 and macrophage markers, formalin-fixed, paraffin-embedded tissues were cut in sections and double-stained with SM α-actin, CD68, CD45, or ABCA1 antibodies. Sections were dewaxed and antigen was retrieved as described above and then coincubated with polyclonal rabbit anti-human SM α-actin (Abcam), mouse monoclonal anti-human SM α-actin (Abcam), monoclonal mouse anti-CD68 antibody (Dako), rabbit polyclonal anti-CD45 antibody (Abcam), mouse anti-CD45 (Dako), or rabbit polyclonal anti-ABCA1 antibody (Novus Biologicals) overnight. AlexaFluor 488–conjugated donkey anti-mouse immunoglobulin G and AlexaFluor 594–conjugated donkey anti-rabbit immunoglobulin G were used for detection. Nuclei were stained by using Hoechst 33342.

Confocal Microscopy

Confocal microscopy was acquired with a Leica AOB5 SP2 laser scanning confocal microscope (Leica, Heidelberg, Germany) by using a high-resolution Leica 63×1.4 Plan-Apochromat oil immersion objective. The acquisition software was Leica Confocal Software TCS SP2. All images and spectral data were captured with the use of photomultiplier tube detectors (R6357; Hamamatsu, Shizuoka-ken, Japan) located inside the scan head. A total of 50 Z-stacks were acquired from the whole section thickness (5 μm) to reconstruct 3-dimensional images. Spectral scanning was performed on each fluorescent dye to confirm its specificity and to ensure that there was no overlap in the emission signal range collected for each fluorescent dye used in this study, as well (data not shown).

Image Analysis

Slides were viewed with the use of a Nikon Eclipse TE300 inverted microscope, illuminated by a Nikon Super High Pressure Mercury Lamp, filtered by using 4′,6-diamidino-2-phenylindole, fluorescein isothiocyanate, or rhodamine filter sets, and captured by using a Spot digital camera (Diagnostic Instruments).

ImagePro Plus 5.1 image analysis software was used to combine 3 obtained images (green, red, and blue) in a single image, named Merge in Figures, for quantification.

Quantification of SMC Foam Cells

The relative contribution of SMC foam cells to total intimal foam cells in human atherosclerotic lesions was determined by 2 independent observers counting foam cells that were strongly positive for or lacking SM α-actin. Quantitation of foam cells was performed by comparing images of the same field of view showing 4′,6-diamidino-2-phenylindole merged with oil red O or SM α-actin immunofluorescence, and applying a grid on each image to reference specific cells between the 2 images. Foam cells were first identified as cells expressing strong oil red O fluorescence, and the expression of SM α-actin in each foam cell was determined by locating it in the corresponding SM α-actin micrograph with the use of the grid. With the use of this method, SM α-actin–positive foam cells were counted for each field and the entire tissue section and expressed as a percentage of total foam cells. Foam cells staining weakly for SM α-actin were not included in the SMC foam cell estimate, but were counted as part of the non-SMC foam cell fraction. There was no statistically significant difference between the percentage of SMC foam cells counted by the first and second observers.

ABCA1 Colocalization

ABCA1 expression in intimal SMCs and myeloid cells was determined by measuring the degree of colocalization between ABCA1 and SM α-actin or CD45, respectively, by using ImagePro Plus 5.1 image analysis software. In brief, areas of interest were selected, separating the intima and media of vessels by using the internal elastic lamina as identified from adjacent sections stained with Movat pentachrome. Color segmentation was used to determine ABCA1, SM
detected using ABCA1 immunoreactivity between SMCs and myeloid-origin cells in advanced lesions. The expression patterns were therefore used to determine the relative contribution of SMCs to total foam cell formation. The expression patterns of actin isoforms including SMα-actin expression array found no evidence of SMα-actin expression by myeloid lineage cells. SMα-actin was therefore used to identify SMCs from myeloid lineage cell–derived foam cells. Specimens from 14 subjects showed foam cells clearly visible in the intima (types II and III atherosclerosis). These 14 specimens were therefore used to determine the relative contribution of SMCs to total foam cell formation. The expression patterns of actin isoforms including SMα-actin in 9783 samples from normal and diseased human specimens by Affymetrix gene expression array found no evidence of SMα-actin expression by myeloid lineage cells. SMα-actin was therefore used to identify SMCs from myeloid lineage cell–derived foam cells. Sections treated first to preserve lipids were stained with SMα-actin followed by oil red O to identify SMC-derived foam cells.

**Table.** Classification of Atherosclerotic Lesions According to Stary et al

<table>
<thead>
<tr>
<th>AHA Classification</th>
<th>Description</th>
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<tbody>
<tr>
<td>Type I (initial)</td>
<td>Accumulation of smooth muscle cells in the intima with isolated macrophage foam cells</td>
</tr>
<tr>
<td>Type II (fatty streak)</td>
<td>Intracellular lipid accumulation/foam cells</td>
</tr>
<tr>
<td>Type III (intermediate)</td>
<td>Type II changes with extracellular lipid pools or small core</td>
</tr>
<tr>
<td>Type IV (atheroma)</td>
<td>Type II changes with obvious core and overlying cap</td>
</tr>
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AHA indicates American Heart Association.
cells (Figure 2). Foam cells either positive or negative for SM α-actin staining were quantitated in multiple images of these sections (subject characteristics, number of images studied, and percentage of SMC foam cells per section are shown in Table I in the online-only Data Supplement). The results showed that 50±7% (average±standard error of the mean, n=14 subjects) of foam cells also costained strongly with SM α-actin. This likely represents an underestimate of SMC contribution to total foam cells, owing to a report of very low or no SM α-actin expression by some intimal SMCs. Overall, these results suggest that the majority of foam cells in human coronary atherosclerosis are SMC rather than myeloid cell derived.

**Expression of ABCA1 Is Lower in Intimal SMCs from Advanced in Comparison With Early Native Atherosclerosis**

We previously reported that ABCA1 expression is reduced in SMCs in the intimal layer in comparison with the medial layer expression by some intimal SMCs. Overall, these results suggest that the majority of foam cells in human coronary atherosclerosis are SMC rather than myeloid cell derived.

**Figure 2.** Identification of SMC foam cells in the intima of human coronary artery atherosclerosis. Immunohistochemical studies were performed with costaining of SM α-actin with horseradish peroxidase–tagged (arterial cross-section, brown) or Alexa 488–tagged (green) secondary antibodies and oil red O (red). Nuclei were stained with DAPI (blue). The merge panel indicates SMC-derived foam cells containing SM α-actin and oil red O. Scale bar, 200 μm. DAPI indicates 4′,6-diamidino-2-phenylindole; SM, smooth muscle; and SMC, smooth muscle cell.

**Figure 3.** ABCA1 and SM α-actin immunoreactivity in the intima of coronary arteries in early and advanced atherosclerosis. A, Coronary artery sections from patients with early (types I and II lesions) and advanced (types III and IV) native atherosclerosis were double-stained with SM α-actin (green) and ABCA1 (red) antibodies. Nuclei were stained with DAPI (blue). B, The extent of colocalization between ABCA1 and SM α-actin in multiple specimens was determined by color segmentation, and normalized to SM α-actin staining area. Scale bar, 500 μm. ABCA1 indicates ATP-binding cassette transporter A1; DAPI, 4′,6-diamidino-2-phenylindole; I, intima; L, lumen; M, media; and SM, smooth muscle.
of human coronary arteries, providing a potential reason for increased SMC foam cell formation in the intima.12 To determine whether the reduction in intimal SMC ABCA1 expression depends on lesion stage, we determined ABCA1 protein levels in the SMCs of early (types I and II) and advanced (types III and IV) atherosclerotic lesions. As shown in Figure 3A and 3B, intimal SMCs in advanced lesions express significantly fewer ABCA1 in comparison with intimal SMCs in early atherosclerosis when normalized to SM α-actin staining.

Expression of ABCA1 is Lower in SMCs Than in Myeloid-Origin Cells in Advanced Atherosclerotic Lesion Intima

To investigate whether the reduction in ABCA1 expression in advanced lesions is unique to SMCs, we also determined ABCA1 expression by myeloid lineage cells in early- and later-stage atherosclerosis. CD45 is expressed in all leukocytes including monocyte-derived macrophages,23 but it is not expressed by SMCs.24–27 We therefore compared ABCA1 expression in SM α-actin– and CD45-expressing cells in early (types I and II) and advanced (types III and IV) lesions. In comparison with the reduction of ABCA1 expression in intimal SMCs (Figure 4A, top), there was no reduction in ABCA1 expression by myeloid lineage cells in advanced lesions (Figure 4A, bottom). Normalization of ABCA1 and SM α-actin costaining to SM α-actin levels in SMCs, or ABCA1 and CD45 costaining to CD45 levels in myeloid lineage cells from multiple early and advanced lesions is shown in Figure 4B. The results show significantly lower ABCA1 expression by SMCs in comparison with myeloid-origin cells, with no loss of ABCA1 expression by myeloid cells in advanced lesions.

Presence of CD68- and SM α-Actin–Positive SMCs in the Intima of Human Coronary Artery Atherosclerosis

Lipid-loaded human and murine SMCs have been shown to express macrophage markers in vitro,13,14 and the presence of intimal cells expressing both SMC and macrophage markers has been reported in human aorta.15 In the current studies, early (types I and II) and advanced stage (types III and IV) human coronary lesions were stained with SM α-actin (SMC-specific) and CD68, typically considered to be a macrophage lineage marker,28,29 to determine the extent of this phenotypic switch in vivo. Our results show the presence of cells expressing both SM α-actin and CD68, especially in advanced lesions (Figure 5A). Cells costaining strongly for both SM α-actin and CD68 were expressed as a percentage of total CD68+ cells. The data indicate that 18±3% (n=9) and 40±6% (n=15) of CD68+ cells express SM α-actin in early and advanced atherosclerotic lesions, respectively (Figure 5B). Colocalization of SM α-actin and CD68 was also examined by using confocal microscopy. Immunoreactivity against SM α-actin and CD68 showed relative colocalization within cells (Figure 5C).
Spectral scanning of the cells expressing both SM α-actin and CD68 also confirmed the presence of the emission signal range for Alexa 488 (SM α-actin) and Alexa 594 (CD68) in single cells (data not shown).

Not All CD68-Positive Cells in Human Atherosclerotic Lesions Are of Myeloid Origin
To further investigate whether CD68-expressing cells in human atheromas are all of myeloid origin, we performed costaining with CD68 and the specific myeloid cell lineage marker CD45. Double-staining of CD45 and CD68 showed that not all of the CD68-positive cells express CD45. The white boxes in Figure 6A through 6C and the confocal image Figure 6D indicate a myeloid-origin cell expressing both CD68 and CD45. The orange boxes in Figure 6A through 6C and confocal image Figure 6E indicate a spindle-shaped cell expressing CD68, but not CD45. We measured the fraction of CD68+CD45− cells in total CD68+ cells in advanced atherosclerosis. The analysis showed that 34±8% (n=11) of CD68+ cells do not express CD45, suggesting they are not of myeloid origin. These findings further support the expression of CD68 by nonmyeloid origin cells in human atherosclerosis and are consistent with our findings of coexpression of CD68 by intimal SMCs.

Discussion
The existence of smooth muscle foam cells in human atherosclerosis has been known for decades,6 but the contribution of SMCs to total foam cells and cholesterol accumulation in the plaque has been unknown. In the current study, we present data
suggesting that a large proportion, at least 50%, of total foam cells in human coronary intimas are derived from SMCs rather than from monocytes. Consistent with this major contribution of SMCs to foam cell formation, we also found a specific reduction in expression of the cholesterol efflux–promoting protein ABCA1 by SMCs between early- and advanced-stage atherosclerotic lesions, which was not observed in myeloid lineage cells. Also consistent with previous reports showing the expression of macrophage proteins by SMCs following lipid loading, we report for the first time that a high percentage of macrophage marker–expressing cells in human coronary intima are SMC rather than myeloid in origin. These combined findings suggest a much larger role than previously realized for intimal SMCs as a site of excess cholesterol accumulation and as a source of macrophage-like cells in human atherosclerotic plaque.

The ability to delineate foam cells for costaining with SMC or myeloid lineage cell markers depended on adapting a previous method that allowed us to distinguish intracellular from extracellular lipid and maintain arterial wall architecture in formalin-fixed tissues for subsequent paraffin embedding and immunohistochemistry. Using this method, we were able to clearly identify the colocalization or absence of SM α-actin with oil red O staining of intracytoplasmic lipids in foam cells. The conclusion that at least 50% of total foam cells are SMC derived owing to SM α-actin staining is based on the assumption that these cells originated as SMCs, rather than being the product of phagocytosis of apoptotic SMCs or SMC debris by myeloid lineage foam cells. Although staining of phagocytosed SM α-actin in monocyte-derived macrophages is a potential limitation of our conclusions, to our knowledge, this has not been reported in the literature, and staining of SM α-actin in early and late endosomes would be expected to be quite weak. To reduce the chance of counting non-SMCs in the SMC foam cell pool, we only included foam cells staining strongly for SM α-actin in this estimate. Another potential confounder is the expression of SM α-actin by myeloid lineage cells. Although it has been shown that cultured macrophages can express SMC markers, including SM α-actin after stimulation with transforming growth factor-β or thrombin, to our knowledge there is no evidence for SM α-actin expression by myeloid lineage cells in human or mouse tissues in vivo.

There is, however, the likelihood that we have not identified all of the SMCs involved in intimal foam cell formation. Gomez et al have recently shown that an epigenetic mark of SMC lineage cells identifies a percentage of SMCs in advanced human coronary atherosclerosis that do not express typical SMC marker genes including SM α-actin. We did not perform this assay in our studies because the efficiency of finding this epigenetic mark in human intimal SMCs has not been determined, nor whether this discreet mark could be seen in cells first processed to stain intracellular lipids. Although it will be important to attempt this method to further define the percentage of intimal foam cells originating as SMCs, the findings of the Owens group suggest that our conclusion that at least 50% of intimal foam cells are SMC derived is a conservative estimate, and the actual contribution of SMCs to the foam cell population may be much larger.

We previously reported a reduction in ABCA1 expression by intimal in comparison with medial coronary artery SMCs. The current finding of reduced ABCA1 protein in late-stage in comparison with early-stage atherosclerotic lesion SMCs, but not myeloid lineage cells, suggests there is a SMC-specific defect in ABCA1 expression in advancing lesions. Reduced intimal SMC ABCA1 expression also provides a plausible explanation for the high percentage of foam cells originating from SMCs. We have also found that the addition of exogenous oxysterols can partially correct ABCA1 expression in cultured intima-type SMCs, suggesting a potential defect in cholesterol trafficking and generation of endogenous oxysterols necessary to activate liver X-receptor–dependent expression of ABCA1 in these cells. Although the exact nature and in vivo correlation of this defect remain to be confirmed, our results showing no loss of ABCA1 expression in myeloid lineage cells across lesion stages suggest that the specific defects in SMC cholesterol
metabolism are a reason for the high amount of cholesterol accumulation in human atherosclerotic plaque.

Previous observations that human aortic intimal SMCs, and cholesterol-loaded human and mouse arterial SMCs, as well, in culture express macrophage markers led us to examine whether SMCs in human coronary lesions also express macrophage markers. We confirmed the presence of SM α-actin+/CD68+ cells in human coronary atherosclerosis, with ≈40% of CD68+ cells in advanced lesions being of SMC origin (Figure 5). Again, our inclusion only of cells staining strongly for SM α-actin reduces the chance that we have included myeloid lineage cells staining for phagocyted SMC fragments; the likely absence of SM α-actin expression by some intimal SMCs suggests that this could also be an underestimate. Consistent with this estimate is our finding that ≈34% of CD68+ cells in advanced lesions do not express CD45, a myeloid cell lineage marker not found to be expressed by SMCs. This further indicates that cells expressing both SM α-actin and CD68 but not CD45 are of SMC rather than myeloid origin. Although the functional significance of SMCs assuming a macrophage phenotype in vivo is not yet known, cultured mouse aortic SMCs converted to a macrophage gene expression pattern following lipid loading also showed an increase in phagocytic activity. Our results along with previous studies indicate that many intimal cells, identified as CD68+ in tissue sections and assumed to be of monocyte or other myeloid cell origin, are in fact SMCs that have converted to a macrophage-like phenotype.

The most striking aspect of these findings is the heightened importance they suggest should be placed on understanding cholesterol metabolism and its dysregulation in intimal SMCs, in addition to intimal monocyte-derived macrophages and other myeloid lineage cells. The perceived lack of importance of intimal SMCs to the composition of plaque in mouse models of atherosclerosis, which the recent Gomez et al studies suggest has been unrecognized because of the loss of classic SMC markers, is a likely reason that there has been so little attention paid to arterial SMC cholesterol metabolism and the role of SMC foam cells in plaque biology. Whether mouse models of atherosclerosis exhibit the same defects in intimal SMC cholesterol metabolism, to support their use in studying this aspect of atherogenesis, remains to be seen.

In conclusion, the current studies suggest a much larger role for intimal SMCs in foam cell formation and overaccumulation of cholesterol in human atherosclerosis than previously assumed. Although myeloid lineage cells express abundant ABCA1 in both early- and late-stage atherosclerosis, the selective decline in ABCA1 expression by later-stage intimal SMCs provides a likely reason for this contribution of SMCs to total foam cell formation, and as a repository for much of the excess cholesterol in the artery wall. The expression of a macrophage marker by intimal SMCs also suggests that many of the intimal macrophages previously assumed to be of monocyte origin are in fact SMCs assuming a macrophage-like state. Further understanding of the mechanisms of intimal SMC cholesterol loading and unloading will open up new targets for the prevention of cholesterol accumulation in the artery wall and its clinical outcomes.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Cholesterol that accumulates in arterial plaque foam cells has previously been assumed to be mainly in macrophages derived from monocytes that migrate into the plaque following endothelial injury. Smooth muscle cells (SMCs) also migrate into arterial intima in the preatherosclerotic stage and during plaque development, and also become foam cells, but the contribution of SMCs to foam cell formation in comparison with monocytes has never been quantified. Using human hearts explanted at the time of transplantation, we found that >50% of the foam cells in human coronary arteries are derived from SMCs rather than monocyte/macrophages and that these SMCs have a selective loss of the cholesterol exporter ATP-binding cassette transporter A1 as lesions advance that is not seen in the monocyte-type cells. This provides a possible explanation for the overaccumulation of cholesterol in these SMC foam cells and in the plaque. We also found that the SMCs in the intima start to express a commonly used marker of macrophage cells, meaning they may be confused for monocyte-derived macrophages in the plaque. Overall, this research shifts our thinking about which cells accumulate cholesterol in the plaque more toward SMCs, and opens up a new direction for research to understand why this occurs in SMCs, and how to prevent it to reduce ischemic vascular events.
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Supplemental Material
Supplemental Table 1. Subject characteristics, number of images studied and percent SMC foam cells per section

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<th>Subject no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Primary diagnosis</th>
<th>Number of images studied</th>
<th>Percent of SMC foam cell</th>
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Supplemental Table 2. Clinical characteristics of patients

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