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

Sima Allahverdian, MD, PhD; Ali Cyrus Chehroudi; 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 of foam cell–rich lesions revealed that 50±7% (average±standard error of the mean, n=14 subjects) of total 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 atherosclerosis8,9 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.10 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.
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 were used for immunohistochemical 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 Biologicals) overnight. AlexaFluor 488–conjugated donkey anti-mouse immunoglobulin G and AlexaFluor 594–conjugated donkey anti-rabbit immunoglobulin G were used to detect SM α-actin and CD45 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 achieved with a Leica AOBS SP2 laser scanning confocal microscope (Leica, Heidelberg, Germany) by using a high-resolution Leica 63X/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...
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Oil red O staining only with no foam cells (type I atherosclerosis, Table). This enhanced lipid fixation technique, 7 showed extracellular lipid staining only with no foam cells (type I atherosclerosis, Table). The extracellular lipid in these sections was localized to the deep intima, as indicated in Figure 1B. In 6 of 7 of these subjects, the intima was also rich in SMCs, with myeloid cells only observed in the superficial intima close to the lumen, as previously reported. Lesions from 10 subjects showed advanced atheromas as indicated by a large necrotic core and fewer intact foam cells (type IV atherosclerosis). 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 SM α-actin in 9783 samples from normal and diseased human specimens by Affymetrix gene expression array found no evidence of SM α-actin expression by myeloid lineage cells, SM α-actin was therefore used to identify SMC from myeloid lineage cell–derived foam cells. Sections treated first to preserve lipids were stained with SM α-actin followed by oil red O to identify SMC-derived foam cells.

### Results

**Detection of Extra- and Intracellular Lipid in Arterial Wall Sections**

To identify the origin of foam cells with the use of cell-specific markers, we first adapted a method to stain lipids by using oil red O in formalin-fixed rather than frozen tissues. This method allows a much clearer distinction between extracellular and intracellular neutral lipids than can be achieved by using standard oil red O staining of frozen tissues. Figure 1B demonstrates extracellular lipids in proximity to SMCs, stains brown by using anti–SM α-actin antibody, much more clearly than can be seen in a similarly stained section by using standard methods for frozen samples (Figure 1A). Figure 1D demonstrates staining of intracellular lipids in foam cells of a more advanced lesion, also with a high degree of clarity in comparison with a frozen section (Figure 1C).

**Quantification of SM α-actin+/CD68+ Cells in Intima**

To determine the proportion of intimal CD68-positive cells expressing SM α-actin, a quantitative immunofluorescence microscopy technique was used. Cells positive for CD68 and positive or negative for SM α-actin immunoreactivity were counted by 2 independent observers using the methods described above, with a slight modification. In brief, a grid was applied to immunofluorescence micrographs showing CD68 counterstained with 4',6-diamidino-2-phenylindole. The expression of SM α-actin in CD68-positive cells was assessed by locating each CD68-positive cell in the matching SM α-actin micrograph. Nucleated cells expressing CD68 only or CD68 and SM α-actin were each totaled, and the percentage of total CD68-positive cells expressing SM α-actin was determined for each section. This measurement was done by 2 observers with 1 blinded to identity of protein markers and lesion stage. Only cells staining strongly positive for both SM α-actin and CD68 were counted as SM α-actin+/CD68+ cells. There was no statistically significant difference between the percentages of SM α-actin+/CD68+ cells counted by the first and second observers.

**Statistical Analysis**

All data are expressed as average±standard error of the mean. Significant differences between 2 independent experimental groups were determined by using 2-tailed Student t tests, with a value of P<0.05 considered significant. Paired t tests were performed to determine statistical differences between the measurements obtained by the first and second observers and ABCA1 immunoreactivity between SMCs and myeloid-origin cells in advanced lesions.

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

<table>
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<th>AHA Classification</th>
<th>Description</th>
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<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>
</tbody>
</table>

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. 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, but it is not expressed by SMCs. 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 co-staining to SM α-actin levels in SMCs, or ABCA1 and CD45 co-staining 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, and the presence of intimal cells expressing both SMC and macrophage markers has been reported in human aorta. 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, 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 co-staining 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, 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
ing a previous method that allowed us to distinguish SMC or myeloid lineage cell markers depended on adapt-
mulation and as a source of macrophage-like cells in human 
artherosclerotic plaque.

realized for intimal SMCs as a site of excess cholesterol accu-
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 
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 per -
centage 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 accumu-
lation and as a source of macrophage-like cells in human 
artherosclerotic 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 colocaliza-
tion 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 iden-
tified all of the SMCs involved in intimal foam cell forma-
tion. 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 fur-
ther 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 expres-
sion 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 advanc-
ing 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, sugges-
ting a potential defect in cholesterol trafficking and gen-
eration 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 myoid lineage cells staining for phagocytosed 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 myoid 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 myoid 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 myoid 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.

Acknowledgments

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Disclosures

None.

References

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 Table 1. Subject characteristics, number of images studied and percent SMC foam cells per section

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### Supplemental Table 2. Clinical characteristics of patients

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<th>Primary diagnosis</th>
<th>Stage of atherosclerosis</th>
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