REVIEW

Apolipoprotein A-I and A-I mimetic peptides: a role in atherosclerosis

Godfrey S Getz
Catherine A Reardon

The University of Chicago,
Department of Pathology,
Chicago, IL, USA

Abstract: Cardiovascular disease remains a major cause of morbidity and mortality in the westernized world. Atherosclerosis is the underlying cause of most cardiovascular diseases. Atherosclerosis is a slowly evolving chronic inflammatory disorder involving the intima of large and medium sized arteries that is initiated in response to high plasma lipid levels, especially LDL. Cells of both the innate and adaptive immunity are involved in this chronic inflammation. Although high plasma LDL levels are a major contributor to most stages of the evolution of atherosclerosis, HDL and its major protein apoA-I possess properties that attenuate and may even reverse atherosclerosis. Two major functions are the ability to induce the efflux of cholesterol from cells, particularly lipid-loaded macrophages, in the artery wall for transfer to the liver, a process referred to as reverse cholesterol transport, and the ability to attenuate the pro-inflammatory properties of LDL. The removal of cellular cholesterol from lipid-loaded macrophages may also be anti-inflammatory. One of the most promising therapies to enhance the anti-atherogenic, anti-inflammatory properties of HDL is apoA-I mimetic peptides. Several of these peptides have been shown to promote cellular cholesterol efflux, attenuate the production of pro-inflammatory cytokines by macrophages, and to attenuate the pro-inflammatory properties of LDL. This latter effect may be related to their high affinity for oxidized lipids present in LDL. This review discusses the functional properties of the peptides and their effect on experimental atherosclerosis and the results of initial clinical studies in humans.

Keywords: apoA-I, mimetic peptides, HDL, anti-inflammatory, atherosclerosis

Introduction

Cardiovascular disease remains a major cause of morbidity and mortality in the westernized world, despite the successes in reducing the outcomes of this disease consequent on the aggressive use of statins, which aim to lower plasma LDL-cholesterol. Atherosclerosis is at the core of the largest proportion of subjects with these diseases. Atherosclerosis is a slowly evolving chronic inflammation involving the intima of large and medium-sized arteries initiated in response to high plasma lipids, especially LDL, and the uptake of modified LDL by macrophages in the artery wall. Components of both the innate and adaptive immune systems are involved.[1–3] Clinically significant atherosclerosis manifests in mid-to-late decades of life, but it is initiated earlier with detectable atherosclerotic lesions present in the second to fourth decades of life.[4] The affected arteries are the carotids, coronaries, aortic arch, renals, and femorals, resulting in stroke, myocardial infarction, renal stenosis, impaired function, and intermittent claudication, respectively. The major risk factors giving rise to atherosclerosis are elevated blood cholesterol (mainly LDL-cholesterol), hypertension, smoking, obesity,

Correspondence: Godfrey S Getz
University of Chicago, Box MC 1089,
5841 South Maryland Avenue,
Chicago, IL 60637, USA
Tel +1 773 834 4856
Fax +1 773 834 5251
Email g-getz@uchicago.edu

diabetes, low HDL cholesterol, and of course age because of the slow evolution of vascular inflammation.[5] Atherosclerotic lesions do not appear uniformly around the circumference of blood vessels. When examined in cross-section, the lesions are usually found on one side of the vessel lumen with the other side relatively lesion-free. This distribution is related to the hemodynamics of blood flow.[6] The blood components most important for the initiation of atherosclerosis are lipoprotein particles, particularly LDL, and leukocytes, mainly monocytes.[2] In regions of laminar flow, these components are in the center of the blood flow in the artery, whereas in low shear stress areas such as at bends and branch points of vessels, these components spend more time in close approximation to the endothelium of the artery wall, the so-called near wall phenomena.[6] Shear stress differences also alter the gene expression pattern of the endothelial cells including changes that promote the influx of leukocytes into the luminal layer of the vessel wall.[6] Although several arterial sites are susceptible to the development of atherosclerosis, the lesions do not develop simultaneously in all sites nor do all individuals develop lesions at all sites. We believe this to be due to subtle differences in the arterial configuration and hence hemodynamic influences which might imprint the underlying endothelial phenotype and affect the response to risk factors.[7]

LDL dysregulation and atherogenesis

Two genetic diseases have been most informative about the role of LDL in human atherosclerosis. First was the finding that humans with familial hypercholesterolemia (FH), especially those with homozygous mutations affecting the function of the LDL receptor, are at significantly increased risk of developing premature atherosclerosis and its clinical sequelae.[8] In the absence of normal LDL receptor function in the liver, plasma and LDL cholesterol are elevated several fold. Brown and Goldstein's interest in understanding the mechanisms responsible for the phenotype in FH patients led them to examine the role of the LDL receptor in the loading of macrophages with cholesteryl ester, an important initial step in atherogenesis. Their studies provided strong evidence for the relatively minor role of the LDL receptor in the loading of macrophages with lipids.[9] Rather they showed that lipid loading of macrophages involved the uptake of modified LDL, not native LDL, by a process involving cell surface scavenger receptors. This observation accounted for the increased atherosclerosis in FH patients and generated important information for the subsequent study

of atherogenesis. This, however, does not belie the critical importance of the LDL receptor in plasma lipoprotein and cholesterol homeostasis and the impact of high plasma LDL levels on atherogenesis. In contrast to those patients with hypercholesterolemia, patients who have reduced levels of plasma LDL as a result of a loss of function of the gene encoding proprotein convertase subtilisin-like/kexin type 9 (PCSK9) develop modest atherosclerosis.[10] PCSK9 is responsible for accelerating the turnover of the LDL receptor.[11] Thus in the absence of fully functional PCSK9, cell surface LDL receptor, particularly that of the hepatocyte, is more abundant and has a longer half-life resulting in lower levels of plasma LDL-cholesterol. As a result these patients are exposed to low plasma LDL levels throughout their lifespan.

Almost all animal models of atherogenesis involve genetic or diet manipulations of plasma lipoprotein levels, particularly LDL.[12] How does persistently elevated plasma LDL-cholesterol promote atherogenesis and how is this related to inflammation?[1–3] With elevated plasma LDL there is an increased ingress of this lipoprotein into the intima of the vessel wall at atherosclerosis susceptible sites, where it is both retained by the matrix proteoglycans and modified either modestly or substantially by oxidation mediated by peroxidase, lipoxygenase, or reactive oxygen species (ROS). The ROS generated in the presence of lipoxygenase results in the oxidation of phospholipids in the LDL, generating oxidized fatty acids. Cholesterol is also oxidized, forming ketocholesterol which is cytotoxic.[13] Modified LDL may be taken up by macrophages through macropinocytosis induced by minimally modified LDL binding to the pattern recognition receptor Toll-like receptor (TLR) 4 or by scavenger receptors expressed on the surface of monocytes that have differentiated into macrophages.[14] Cholesterol loading of macrophages has a substantial impact on networks of genes that are either up- or downregulated.[15] If there is sufficient uptake of modified LDL, the lipoprotein cholesterol deposits in cytoplasmic droplets primarily as cholesteryl esters producing the foam cell which is the cardinal cell involved in the early stages of atherosclerosis. The uptake of modified lipoprotein or their interaction with cell surface receptors also results in the activation of a variety of signaling pathways in the macrophage leading to the production of cytokines, chemokines, growth factors, and ROS that influence the overlying endothelial cells and the smooth muscle cells in the vessel wall directly or by further modification of LDL which can also induce signaling in endothelial cells. The result of these regulatory phenomena is the production of chemotactic factors such as monocyte chemotactic protein 1 (MCP-1) which helps in the

recruitment of the monocytes into the intima, the production of adhesion molecules such as VCAM1 and ICAM1, and the secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF) which promotes the local differentiation of monocytes to macrophages. Many of these proteins have an impact not only on the behavior of macrophages, but also the recruitment of other inflammatory cells. The growth factors promote the migration of smooth muscle cells from the arterial media into the intima, where they synthesize matrix proteins and are also responsible for the formation of the fibrous cap which overlies the maturing plaque. Other cells are recruited from the blood stream, most notable are T cells. This includes T helper 1 (T$_H$1) cells which secrete pro-inflammatory cytokines such as interferon γ (IFNγ), and T regulatory (Treg) cells which secrete anti-inflammatory cytokines such as IL-10 and transforming growth factor β (TGFβ). The natural killer T cell subsets have also been implicated in atherogenesis. These T cell cytokines may influence gene expression in the macrophages of the lesions, including the expression of costimulatory molecules which participate in the activation of the T cell subsets. Other minor components of the innate and adaptive immune system have also been implicated in the evolution of atherosclerosis.[16]

Oxidized lipids in the modified LDL produced by the lipoxygenase pathway can couple with the lysine residues of apoB, the major apoprotein in LDL.[13] These changes create neo-antigens in the LDL which can activate the innate and adaptive immune system, generating antibodies that may be protective against the further evolution of atherosclerosis and implicating the participation of B cells in atherogenesis.[17] The elicited immune reaction has led to studies of immunization approaches to the treatment of atherosclerotic disease mostly using apoB peptides as antigen.[18,19] The results of these experimental studies show considerable promise.

As atherosclerosis proceeds, lipid-loaded macrophages may undergo apoptosis or necrosis. With cholesterol loading there is an increase in both cholesteryl ester and free cholesterol with the latter eliciting the unfolded protein response and the activation of the apoptotic cascade.[20] Apoptotic macrophages may be phagocytosed by surrounding phagocytes, a process designated as efferocytosis. When apoptotic cells are efficiently removed, this promotes inflammation resolution and lesions decrease in size; on the other hand, when efferocytosis is inefficient, apoptotic macrophages accumulate and may undergo secondary necrosis, creating the necrotic core of advancing atherosclerotic plaques and increasing vascular inflammation.[21] These cores may include proteolytic enzymes and sufficient cholesterol to crystallize.

The proteolytic enzymes may play a role in the disruption of the overlying fibrous cap of the atherosclerotic lesion made by smooth muscle cells which have migrated from the media. The lesions containing necrotic cores are less stable and may result in the formation of an acute atherothrombosis and possible embolization giving rise to the critical clinical complications of atherosclerotic vascular disease.

The above discussion highlights the importance of LDL and cholesterol in various stages of the vascular inflammation associated with atherosclerosis. Statin drugs inhibit 3-hydroxy-3-methyl-glutaryl-CoA (HMG CoA) reductase and efficiently upregulate the production of the LDL receptor, thus lowering plasma LDL levels, and are one of the most widely prescribed treatments for hypercholesterolemia. Despite the success of statin treatment in lowering the clinical outcomes in atherosclerotic disease, cardiovascular disease remains the major cause of morbidity and mortality in the westernized world. One of the reasons is probably attributable to the fact that statin treatment is usually initiated in mid-to-late life when atherosclerotic lesions are quite well advanced. The atherosclerotic plaque, though a chronic inflammatory reaction, is a dynamic entity and potentially capable of undergoing a "reversal". The most dynamic cell component of the atherosclerotic plaque is likely the lipid-loaded foam cell.[22] Statins alone seldom lower LDL-cholesterol in human studies to levels that induce substantial reversal of existing atherosclerotic lesions. Statins probably function mainly by limiting the progression of atherosclerotic lesions.

HDL as an atheroprotective agent

The continuing morbidity and mortality of cardiovascular disease in the face of widespread statin treatment has led to a focus on the HDL component of the plasma. There is a great deal of epidemiological and experimental animal evidence suggesting that HDL is protective against atherosclerosis and its sequelae.[23,24] HDL is also thought to be the key player in the potential reversal of atherosclerosis.[25,26] Several subclasses of HDL exist in humans which are differentiated by size and density. As its name suggests HDL is denser than LDL, carrying a larger proportion of protein to lipids than LDL. The major apoproteins of HDL are apoA-I and apoA-II. However the recent proteomic analysis of human HDL indicates that many other proteins may be associated with this lipoprotein in subparticle stoichiometry.[27,28] This suggests that when the minor proteins are taken into account, HDL exhibits a very large degree of microheterogeneity. However the precise function of many of these substoichiometric components of HDL

is unknown. Most attention has been focused on apoA-I since it is the major apoprotein in HDL, constituting about two-thirds of the total HDL protein and is probably a major component of each HDL particle. In humans, the mature protein contains 243 amino acids with the last 199 amino acids of the protein composed of a series of ten repeating amphipathic α-helices.[29] Eight of these helices have 22 amino acids and two have 11 amino acids. The amphipathic α-helices are class A helices in which hydrophobic residues occupy one face whereas neutral and negatively charged residues occupy the other (polar) face with the positively charged lysine and arginine residues found between the two faces of the helix.[30] This general structure of apoA-I is highly conserved among essentially all mammalian species.[30] A major and much studied function of HDL and apoA-I is the promotion of reverse cholesterol transport involving the transfer of cholesterol from peripheral tissue, including the macrophage foam cells of the atherosclerotic lesions, through the plasma to the liver whence it is excreted either as free cholesterol or bile salt into the feces.[31] Two major pathways involving ABC transporters have been identified for the efflux of cholesterol from lipid-loaded macrophage foam cells to HDL or apoA-I. Intact HDL acquires cellular cholesterol via interacting with ABCG1, while lipid-poor apoA-I, which can be liberated from remodeled HDL, is able to catalyze the removal of cellular cholesterol via ABCA1.[32,33] The importance of this latter transporter is indicated by the phenotype of patients with Tangier disease caused by a deficiency of ABCA1.[34] These patients have virtually no HDL and exhibit highly lipid-enriched foam cells in the reticuloendothelial tissues, eg, tonsils.

Although much attention has been devoted to the role of HDL and apoA-I in mediating reverse cholesterol transport, it is now becoming more widely recognized that HDL has other functions that could potentially influence atherosclerosis. These include the inhibition (or attenuation) of LDL oxidation, inhibition of type 1 interferon production as a consequence of bacterial infection, inhibition of chemokine production particularly CCL2 (MCP-1) and CCL5 which may be responsible for the recruitment of cells into the atherosclerotic plaque, and promotion of the stability of endothelial nitric oxide synthase (eNOS) which allows for vascular dilation.[35–41] Given that diabetes may significantly contribute to the risk of atherosclerosis and cardiovascular disease, the recent observation that the HDL may promote insulin secretion by pancreatic islets is of great interest.[42]

HDL has additional anti-inflammatory properties. It has long been recognized that HDL binds LPS, thus blocking its ability to trigger signaling via the CD14/MD2/TLR4 complex.[43]

More recently it has been shown that HDL and/or apoA-I binding to ABC transporters on macrophages is anti-inflammatory. One indirect mechanism appears to be related to the promotion of lipid efflux thus reducing cell surface rafts leading to reduced signaling via TLR4 and perhaps other TLRs.[44,45] Another mechanism appears to be due to apoA-I mediated stabilization of ABCA1 resulting in the autophosphorylation of JAK2 and activation of STAT3 which promotes an anti-inflammatory phenotype in the macrophages.[46,47] This reduced expression of pro-inflammatory cytokines (IL-1, IL-6, and TNFα) by macrophages induced by apoA-I is independent of the lipid transport activity of ABCA1.[47]

Although a great deal of attention has been paid to the measurement of the level of plasma HDL and to the development of therapeutics to increase HDL levels, it is now becoming increasingly recognized that the functional attributes of HDL may be as important or perhaps even more important than the level in the plasma. One assessment of the function of HDL has been based upon an assay developed by Navab et al.[48] In this assay, human endothelial cells cocultured with smooth muscle cells are incubated with plasma LDL. The endothelial cell mediated oxidation of the LDL induces the production of MCP-1 by the endothelial cells. Upon the addition of monocytes to the coculture, the MCP-1 promotes chemotaxis of the monocytes across the monolayer of endothelial cells. This assay has been designated the "monocyte chemoattractant assay" (MCA) and is dependent upon the oxidation of LDL rendering it pro-inflammatory. The addition of HDL to the system reduces the recruitment of monocytes by lowering the oxidation of LDL, probably in major part by extracting oxidized lipids from LDL, and thus the production of the chemoattractant.[48] HDL needs to be constantly present in the culture medium to be effective. This assay has been used to assign an anti-inflammatory index to different HDL preparations. For example, HDL derived from patients with clinically active cardiovascular disease exert less anti-inflammatory activity than the HDL from control subjects.[49] One mechanism by which HDL may exert this anti-inflammatory action is attributable to its carriage of enzymes capable of cleaving oxidized phospholipids–paraoxonase and platelet activating factor hydrolase.[50] ApoA-I also inhibits the oxidation of LDL, but only if it is removed from the coculture system prior to the addition of monocytes.[51]

The apoA-I mimetic peptides

One of the promising potential therapeutics for improving the function of HDL is apoA-I mimetic peptides.[52] The design

of the apoA-I mimetic peptides is based upon the general structure of the amphipathic α-helical repeats in apoA-I with its separation of polar and nonpolar residues on the two faces of the helix and distribution of charged residues. The first of these peptides was designed by Anantharamaiah et al as an 18 amino acid peptide with the general properties of the average amphipathic helix of apoA-I without reproducing the exact amino acid sequence of any single amphipathic helix of the protein.[53] The peptide, originally named 18A, had 18 amino acid residues rather than the 22 amino acids of the typical helix because it did not include the residues in the junctions between the tandem helices in the protein. Upon acetylation of its N-terminus and amidation of its C-terminus, its helicity and capacity to associate with lipids is increased. This modified peptide is referred to as 2F since it contains two phenylalanine residues on the hydrophobic face (Figure 1). A tandem helix has been studied, which comprises two 2F peptides linked by a proline residue (37pA), the prototypic linking residue between many of the repeating helices of apoA-I. Both peptides efficiently promote cholesterol efflux from cholesterol enriched cells.[54] These peptides are also capable of solubilizing phospholipid micelles, demonstrating their affinity for lipid.[55,56]

A series of 18 amino acid peptide variants has been fabricated by manipulating the hydrophobic residues of the 2F

peptide by varying the number of phenylalanine residues on the nonpolar face and are named based on the number of phenylalanines in the peptide. The most widely studied of these peptides is 4F which has an additional two phenylalanine residues incorporated into the hydrophobic face in place of leucine residues in 2F at positions 3 and 14 (Figure 1). Thus, 4F has phenylalanine residues at four of the eight positions on the nonpolar face (at positions 3, 6, 14, and 18). This peptide has been shown to efficiently promote cellular cholesterol efflux, have high lipid affinity, and is anti-inflammatory in the MCA.[55,57] Other phenylalanine variants that have been studied are: 5F (with phenylalanines at positions 6, 11, 14, 17, and 18), 6F (with phenylalanines at positions 6, 10, 11, 14, 17, and 18), and 7F (with phenylalanines at 7 of the 8 positions). 5F and 6F are as active as 4F in the MCA whereas the biological activity of 2F and 7F is somewhat lower.[55]

A series of peptides containing three phenylalanines located at different positions on the nonpolar face has also been studied. The aromatic residues in 3F-1 are close to the edges of the nonpolar face near the Lys residues, near the center of the nonpolar face in 3F-2, and asymmetrically aligned on the nonpolar face in 3F[14] (Figure 1). 3F-1 and 3F-2 are highly active biologically in the MCA in contrast to another variant 3F[14].[58,59] An examination of the position of the phenylalanine residues on the nonpolar face does not suggest
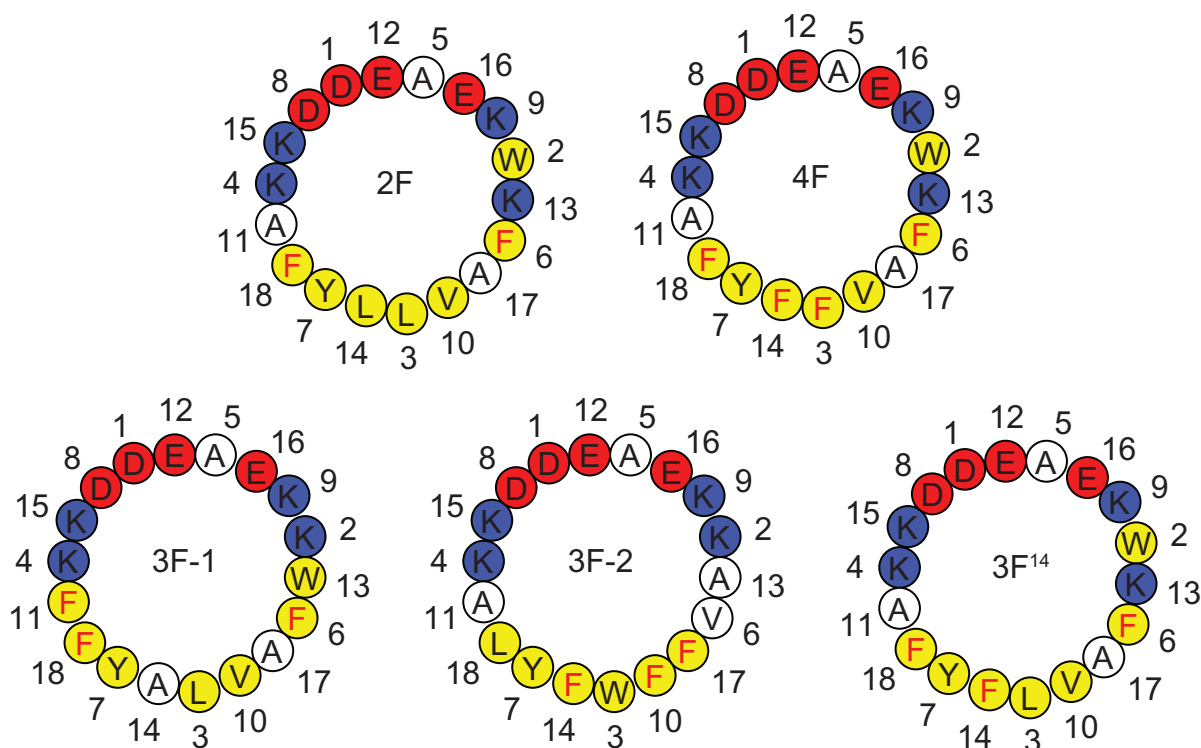

Figure 1 Helical wheel depiction of the several apoA-I mimetic peptide. Hydrophobic residues are yellow, acidic residue are red, and basic residues are blue. The phenylalanine residues are highlighted in red lettering.

a precise structure-function activity of these peptides based upon the phenylalanine position. The bioactive peptides 3F-1 and 3F-2 have larger hydrophobic face than the inactive 3F[14]. On the other hand, the capacity to solubilize palmitoyl oleoyl phosphatidyl choline (POPC) phospholipid micelles does not correlate with bioactivity: 3F[14] is more effective than either 3F-1 or 3F-2, indicating that other factors than hydrophobicity and lipid affinity are important for their anti-inflammatory properties (Table 1).[58,59] Correlated with bioactivity is the motion of the tryptophan residues in the peptides.[58,59] In 3F[14], this residue is more spatially restricted than is the case for 4F, 3F-1 or 3F-2.

The anti-inflammatory properties of apoA-I mimetic peptides

One of the properties of the mimetic peptide that initially excited interest was their anti-inflammatory activity in the monocyte chemotactic assay described above. Like apoA-I, the apoA-I mimetic peptides are able to inhibit LDL-mediated stimulation of monocyte chemotactic activity. But the peptides differ from apoA-I in that while apoA-I has to be removed from the culture system prior to the addition of monocytes in order to reduce the inflammatory activity of the LDL, the peptide can continuously remain in the assay media.[51,60] This difference in apoA-I and mimetic peptide behavior is probably attributable to a difference in their interaction with oxidized lipids. Van Lenten et al have shown that the mimetic peptides that are anti-inflammatory in the MCA and attenuate atherosclerosis in animal models (ie, 4F, 3F-1, and 3F-2) have a very high affinity for oxidized fatty acids and cholesterol as well as oxidized phospholipids.[60] This affinity is ~4–6 orders of magnitude higher than that of apoA-I. The binding of oxidized lipids to the less biologically active mimetic peptide 3F[14] is significantly lower. This has led to the hypothesis that the anti-inflammatory activity of the peptides is related to their ability to sequester oxidized lipid. Consistent with this is the lower levels of oxidized fatty acids in the plasma of mice

treated with 4F.[61] The anti-inflammatory activity of HDL is probably attributable to the retention of oxidized lipids in the lipid domain without exhibiting the high chemical affinity for these lipids that the peptides do. The peptides are also able to reverse the pro-inflammatory activity of the dysfunctional HDL obtained from patients with clinical atherosclerotic disease.[62] This is probably attributable to the extraction of oxidized lipids from the HDL.

One of the properties in which the peptides resemble HDL and apoA-I is their ability to promote reverse cholesterol transport. 2F, 4F, and the stereochemical isomer of 2F synthesized with D amino acids (D2F) are capable of promoting cholesterol efflux from cholesterol loaded macrophages and stabilizing ABCA1.[46] In addition to interacting with ABCA-1, the peptides probably also interact with membrane lipids which may be of importance in promoting efflux. We have studied tandem peptides consisting of two monomeric 4F peptides linked by a proline or with a seven amino acid interhelical sequence. They also promote cholesterol efflux as effectively as the monomeric 4F.[57] The 4F peptide has been shown to promote reverse cholesterol transport in vivo using an assay involving the injection of [³H]-cholesterol labeled, lipid load J774 macrophage cell line into the peritoneal cavity of mice.[63] Whether tandem peptides are more efficient has not yet been determined.

Similar to apoA-I, 2F, 4F, and D2F are capable of activating the autophosphorylation of JAK2.[46] Whether phosphorylation of Stat3 upon binding to ABCA1 is a property that peptides share with apoA-I is yet to be established. However, it has been shown that like apoA-I, 4F reduces lipid rafts in macrophages and this is correlated with decreased production of pro-inflammatory cytokines and chemokines and cell surface expression of TLR4.[45] The suggestion is that the reduction in lipid rafts is due to removal of cholesterol which in turn modulates the activity of raft associated signaling proteins.

The mimetic peptides have been studied for only some of the other functions of HDL and apoA-I mentioned above.

Table 1 In vivo and in vitro properties of active apoA-I mimetic peptides. Comparison of the anti-inflammatory activity of the peptides in the monocyte chemotactic assay and effects on early murine atherosclerosis are compared with their ability to solubilize the phospholipid POPC and motion of the single tryptophan residue in the peptides. The symbols represent relative levels of activity with +++ the highest and − being no activity

	Anti-inflammatory activity in vitro	Attenuation of early atherosclerosis	Phospholipid solubilization	Tryptophan motion
3F-1	++	nd	+	++
3F-2	++	++	++	++
3F[14]	−	+	+++	+
4F	++	++	++	++

Abbreviations: nd, no data; POPC, palmitoyl oleoyl phosphatidyl choline.

One function that the peptides share with apoA-I is the ability to stimulate eNOS activity and thus facilitating vasodilation of blood vessels.[64, 65] We have recently observed that treatment of mice with 4F leads to an increment in the level of the natural antibody EO6/T15 that recognizes oxidized phospholipids.[66] Antibodies recognizing oxidized phospholipids and other oxidation epitopes may attenuate atherosclerosis.[67,68]

An asymmetrical tandem peptide has been developed by Remaley and colleagues. The N-terminal half of this peptide consists of 2F and the C-terminal half consists of a modified 2F in which each of the hydrophobic residues on the nonpolar face of the helix (positions 3, 6, 10, 14, and 18) were substituted with an alanine residue.[69] Thus, the lipid affinities of two halves of the tandem peptide, designed 5A, are not identical. The ability of 5A to promote cholesterol efflux is less than that of 37pA at low concentration but is more active at high concentrations.

A variety of other peptides based upon the C-terminal helices of apoA-I and the structure of other HDL-associated proteins, ie, apoE, apoJ, and SAA, have also been studied for biological activity in some of the above assays. Some of these studies have been summarized in a recent review.[70] It is not clear that these peptides all have the capacity to bind cytotoxic lipids. The apoE and SAA peptides almost certainly act at least in part by promoting reverse cholesterol transport and in this way are anti-inflammatory.

The in vivo anti-inflammatory activities of the apoA-I mimetic peptides

A great deal of interest was elicited in 2002 when the anti-atherogenic effect of D4F at very low dose was first noted.[71] The peptide was synthesized from D2F to render it resistant to proteolysis by intestinal and bacterial enzymes when administered orally. Since then, many studies have been undertaken to examine the effect of the 4F peptide and other mimetic peptides in experimental atherosclerosis, predominantly in murine models (see [70] for a summary of the studies). Peptides have been administered orally, intravenously, and subcutaneously. When the latter two routes are employed, 4F containing L-amino acids is active. In general, these peptides are most effective at reducing early murine lesions, however, there is one report of the 4F peptide attenuating the development of more mature lesions when peptide treatment was coupled with statin treatment.[72] The effect of larger doses of peptide as a monotherapy on more developed lesions has recently been reported.[73] In addition to the influence on atherosclerotic lesions, these studies are often associated with a reduction in plasma lipid peroxides.[63] In general, peptides with anti-inflammatory properties are also more effective at attenuating atherosclerosis (Table 1). While the theory that the peptides attenuate atherosclerosis mainly by binding pathogenic oxidized lipids is certainly attractive, whether this mechanism is the primary contributor to peptide efficacy in vivo has not yet been categorically established.

The binding of oxidized lipids at high affinity by the active peptides suggested they may be useful agents for the treatment of other inflammatory diseases in which oxidized lipids are thought to be contributing participants. Thus the peptides have been shown to exert anti-inflammatory activity in a variety of inflammatory disorders including viral infection, common asthma, renal inflammation, diabetes, scleroderma, and sickle cell disease,[74,75] and more recently in murine models of chronic arthritis, endotoxemia, and lupus erythematosis.[76–78] Interestingly, in the case of lupus related accelerated atherosclerosis, the 4F peptide in combination with statin did not reduce lesion size but the composition of the lesions was altered with a reduction in macrophage content and an increase in smooth muscle cell content. This is compatible with reduced inflammation. Most of the atherosclerosis and inflammatory disease studies have been performed with the apoE-deficient mouse model in which the oxidative stress is higher than in other models.

Human subjects with clinical atherosclerotic disease biopsies

The anti-atherosclerotic action of the mimetic peptides in preclinical studies has made them very attractive potential agents for clinical therapy, especially in view of their apparent synergy with statins in mouse models. Two human studies have so far been performed. The first was a safety study in which a single oral dose of the peptide D4F was given in the range 4–8 mg/kg.[79] This level of peptide was able to substantially improve the inflammatory index in the HDL of patients with clinical cardiovascular disease without any toxic effects observed. On the other hand, a more recent study by Watson et al in which approximately 1/10 of the above dose of peptide was given daily intravenously over 7 days or subcutaneously for 28 days was without effect on the biomarkers of HDL function.[80] This was the outcome despite the fact that the maximum plasma concentration of peptide was substantially higher than was achieved with a single oral dose study reported by Bloeden et al.[79] In an attempt to understand the differences in the outcome measures of peptide administration in these two human studies, Navab et al have

recently reported an extensive comparison of the effects of various dose of peptides given either orally or subcutaneously to apoE-deficient mice on inflammatory markers including the HDL inflammatory index and atheroslerosis.[73] They found that the dose of peptide, irrespective of the method of administration, was the primary determinant of peptide efficacy, not the level of peptide in the plasma or liver. Clearly, much more work is needed to establish optimal dosing, route of administration, and cotherapies for the treatment of human atherosclerotic heart disease and other chronic inflammatory disorders using these agents.

Conclusion and perspectives

There is much evidence to suggest powerful anti-atherogenic, anti-inflammatory properties of HDL. But therapies targeted at elevating a functionally effective HDL are not far advanced. Given the heterogeneity of HDL, this will not be easily accomplished. HDL promotes reverse cholesterol transport and is anti-inflammatory, two potentially correlated functions. The apoA-I mimetic peptide is a much simpler reagent for therapeutic purposes than HDL or apoA-I. It too may promote reverse cholesterol transport (not yet well studied in vivo) and be markedly anti-inflammatory, perhaps mainly by binding oxidized fatty acids and phospholipids and/or interaction with ABCA1. But there is a need for much more basic pharmacology and pharmacodynamic studies in animals and humans.

- For effective therapy, new modes of peptide delivery need to be explored using such devices as subcutaneously implanted pumps for constant and regular delivery of peptide to the plasma and active tissues.
- More precise experiments on the in vivo mechanisms of action of the mimetic peptides are needed to ascertain what requirements are associated with effective in vivo therapy.
- Given the observation that peptide therapy in animal models may increase the level of antibodies to oxidized phospholipid, one wonders whether these peptides might be employed as a means of antigen presentation of oxidized phospholipid to generate antibodies for the removal of oxidized phospholipids. This needs to be more intensively explored.

Acknowledgments

Disclosure

References

1. Packard RR, Lichtman AH, Libby P. Innate and adaptive immunity in atherosclerosis. *Semin Immunopathol*. 2009;31:5–22.
2. Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol*. 2011;12:204–212.
3. Getz GS, Vanderlaan PA, Reardon CA. The immune system and murine atherosclerosis. *Curr Drug Targets*. 2007;8:1297–1306.
4. McGill HC Jr, McMahan CA. Determinants of atherosclerosis in the young. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. *Am J Cardiol*. 1998;82:30T–36T.
5. McGill HC Jr, McMahan CA, Gidding SS. Preventing heart disease in the 21st century: implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. *Circulation*. 2008;117:1216–1227.
6. Chiu JJ, Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. *Physiol Rev*. 2011;91:327–387.
7. VanderLaan PA, Reardon CA, Getz GS. Site specificity of atherosclerosis: site-selective responses to atherosclerotic modulators. *Arterioscler Thromb Vasc Biol*. 2004;24:12–22.
8. Goldstein JL, Brown MS. The low-density lipoprotein pathway and its relationship to atherosclerosis. *Ann Rev Biochem*. 1977;46:897–930.
9. Goldstein JL, Ho YK, Basu SK, Brown MS. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc Natl Acad Sci U S A*. 1979;76:333–337.
10. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med*. 2006;354:1264–1272.
11. Horton JD, Cohen JC, Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. *J Lipid Res*. 2009;50 Suppl:S172–S177.
12. Getz GS, Reardon CA. Diet and murine atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2006;26:242–249.
13. Navab M, Anantharamaiah GM, Reddy ST, et al. The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. *J Lipid Res*. 2004;45:993–1007.
14. Miller YI, Choi SH, Fang L, Tsimikas S. Lipoprotein modification and macrophage uptake: role of pathologic cholesterol transport in atherogenesis. *Subcell Biochem*. 2010;51:229–251.
15. Becker L, Gharib SA, Irwin AD, et al. A macrophage sterol-responsive network linked to atherogenesis. *Cell Metab*. 2010;11:125–135.
16. Galkina E, Ley K. Leukocyte influx in atherosclerosis. *Curr Drug Targets*. 2007;8:1239–1248.
17. Chou MY, Hartvigsen K, Hansen LF, et al. Oxidation-specific epitopes are important targets of innate immunity. *J Intern Med*. 2008;263:479–488.
18. Klingenberg R, Lebens M, Hermansson A, et al. Intranasal immunization with an apolipoprotein B-100 fusion protein induces antigen-specific regulatory T cells and reduces atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2010;30:946–952.
19. Fredrikson GN, Björkbacka H, Söderberg I, Ljungcrantz I, Nilsson J. Treatment with apoB peptide vaccines inhibits atherosclerosis in human apo B-100 transgenic mice without inducing an increase in peptide-specific antibodies. *J Intern Med*. 2008;264:563–570.
20. Feng B, Yao PM, Li Y, et al. The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nat Cell Biol*. 2003;5:781–792.
21. Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. *Nat Rev Immunol*. 2010;10:36–46.
22. Llodrá J, Angeli V, Liu J, et al. Emigration of monocyte-derived cells from atherosclerotic lesions characterizes regressive, but not progressive, plaques. *Proc Natl Acad Sci U S A*. 2004;101:11779–11784.

23. Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. *Circulation*. 1989;79:8–15.

24. Plump AS, Scott CJ, Breslow JL. Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in apolipoprotein E-deficient mice. *Proc Natl Acad Sci U S A*. 1994;91:9607–9611.

25. Rong JX, Li J, Reis ED, et al. Elevating high-density lipoprotein cholesterol in apolipoprotein E-deficient mice remodels advanced atherosclerotic lesions by decreasing macrophage and increasing smooth muscle cell content. *Circulation*. 2001;104:2447–2452.

26. Tangirala RK, Tsukamoto K, Chun SH, Usher D, Puré E, Rader DJ. Regression of atherosclerosis induced by liver-directed gene transfer of apolipoprotein A-I in mice. *Circulation*. 1999;100:1816–1822.

27. Vaisar T, Pennathur S, Green PS, et al. Shotgun proteomics implicates protease inhibition and complement activation in the anti-inflammatory properties of HDL. *J Clin Invest*. 2007;117:746–756.

28. Gordon SM, Deng J, Lu LJ, Davidson WS. Proteomic characterization of human plasma high density lipoprotein fractionated by gel filtration chromatography. *J Proteome Res*. 2010;9:5239–5249.

29. Li WH, Tanimura M, Luo CC, Datta S, Chan L. The apolipoprotein multigene family: biosynthesis, structure, structure-function relationships, and evolution. *J Lipid Res*. 1988;29:245–271.

30. Bashtovyy D, Jones MK, Anantharamaiah GM, Segrest JP. Sequence conservation of apolipoprotein A-I affords novel insights into HDL structure-function. *J Lipid Res*. 2011;52:435–450.

31. van der Velde AE. Reverse cholesterol transport: from classical view to new insights. *World J Gastroenterol*. 2010;16:5908–5915.

32. Fitzgerald ML, Mujawar Z, Tamehiro N. ABC transporters, atherosclerosis and inflammation. *Atherosclerosis*. 2010;211:361–370.

33. Yvan-Charvet L, Wang N, Tall AR. Role of HDL, ABCA1, and ABCG1 transporters in cholesterol efflux and immune responses. *Arterioscler Thromb Vasc Biol*. 2010;30:139–143.

34. Hayden MR, Clee SM, Brooks-Wilson A, Genest J Jr, Attie A, Kastelein JJ. Cholesterol efflux regulatory protein, Tangier disease and familial high-density lipoprotein deficiency. *Curr Opin Lipidol*. 2000;11:117–122.

35. Feig JE, Shamir R, Fisher EA. Atheroprotective effects of HDL: beyond reverse cholesterol transport. *Curr Drug Targets*. 2008;9:196–203.

36. deGoma EM, deGoma RL, Rader DJ. Beyond high-density lipoprotein cholesterol levels evaluating high-density lipoprotein function as influenced by novel therapeutic approaches. *J Am Coll Cardiol*. 2008;51:2199–2211.

37. Navab M, Reddy ST, Van Lenten BJ, Fogelman AM. HDL and cardiovascular disease: atherogenic and atheroprotective mechanisms. *Nat Rev Cardiol*. 2011;8:222–232.

38. Tölle M, Pawlak A, Schuchardt M, et al. HDL-associated lysosphingolipids inhibit NAD(P)H oxidase-dependent monocyte chemoattractant protein-1 production. *Arterioscler Thromb Vasc Biol*. 2008;28:1542–1548.

39. Suzuki M, Pritchard DK, Becker L, et al. High-density lipoprotein suppresses the type I interferon response, a family of potent antiviral immunoregulators, in macrophages challenged with lipopolysaccharide. *Circulation*. 2010;122:1919–1927.

40. Bursill CA, Castro ML, Beattie DT, et al. High-density lipoproteins suppress chemokines and chemokine receptors in vitro and in vivo. *Arterioscler Thromb Vasc Biol*. 2010;30:1773–1778.

41. Mineo C, Yuhanna IS, Quon MJ, Shaul PW. High density lipoprotein-induced endothelial nitric-oxide synthase activation is mediated by Akt and MAP kinases. *J Biol Chem*. 2003;278:9142–9149.

42. Fryirs MA, Barter PJ, Appavoo M, et al. Effects of high-density lipoproteins on pancreatic beta-cell insulin secretion. *Arterioscler Thromb Vasc Biol*. 2010;30:1642–1628.

43. Levine DM, Parker TS, Donnelly TM, Walsh A, Rubin AL. In vivo protection against endotoxin by plasma high density lipoprotein. *Proc Natl Acad Sci U S A*. 1993;90:12040–12044.

44. Yvan-Charvet L, Welch C, Palger TA, et al. Increased inflammatory gene expression in ABC-transporter-deficient macrophages. Free cholesterol accumulation, increased signaling via Toll-like receptors, and neutrophils infiltration of atherosclerotic lesions. *Circulation*. 2008;118:1837–1847.

45. Smythies LE, White CR, Maheshwari A, et al. Apolipoprotein A-I mimetic 4F alters the function of human monocyte-derived macrophages. *Am J Physiol Cell Physiol*. 2010;298:C1538–C1548.

46. Tang C, Vaughan AM, Anantharamaiah GM, Oram JF. Janus kinase 2 modulates the lipid-removing but not protein stabilizing interactions of amphipathic helices with ABCA1. *J Lipid Res*. 2006;47:107–114.

47. Tang C, Liu Y, Kessler PS, Vaughan AM, Oram JF. The macrophage cholesterol exporter ABCA1 functions as an anti-inflammatory receptor. *J Biol Chem*. 2009;284:32336–32343.

48. Navab M, Imes SS, Hama SY, et al. Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. *J Clin Invest*. 1991;88:2039–2046.

49. Ansell BJ, Navab M, Hama S, et al. Inflammatory/anti-inflammatory properties of high-density lipoprotein distinguish patients from control subjects better than high-density lipoprotein cholesterol levels and are favorably affected by simvastatin treatment. *Circulation*. 2003;108:2751–2756.

50. Podrez EA. Anti-oxidant properties of high-density lipoprotein and atherosclerosis. *Clin Exp Pharmacol Physiol*. 2010;37:719–725.

51. Navab M, Hama SY, Anantharamaiah GM, et al. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. *J Lipid Res*. 2000;41:1495–1508.

52. Degoma EM, Rader DJ. Novel HDL-directed pharmacotherapeutic strategies. *Nat Rev Cardiol*. 2011;8:266–277.

53. Anantharamaiah GM, Jones JL, Brouillette CG, et al. Studies of synthetic peptide analogs of the amphipathic helix. Structure of complexes with dimyristoyl phosphatidycholine. *J Biol Chem*. 1985;260:10248–10255.

54. Yancey PG, Bielicki JK, Johnson WJ, et al. Efflux of cellular cholesterol and phospholipid to lipid-free apolipoproteins and class A amphipathic peptides. *Biochemistry*. 1995;34:7955–7965.

55. Datta G, Chaddha M, Hama S, et al. Effects of increasing hydrophobicity on the physical-chemical and biological properties of a class A amphipathic helical peptide. *J Lipid Res*. 2001;42:1096–1104.

56. Mishra VK, Palgunachari MN, Lund-Katz S, et al. Effect of the arrangement of tandem repeating units of class A amphipathic a-helixes on lipid interactions. *J Biol Chem*. 1995;270:1602–1611.

57. Wool GD, Reardon CA, Getz GS. Apolipoprotein A-I mimetic peptide helix number and helix linker influence potentially anti-atherogenic properties. *J Lipid Res*. 2008;49:1268–1283.

58. Datta G, Epand RF, Epand RM, et al. Aromatic residue position on the nonpolar face of class A amphipathic helical peptides determines biological activity. *J Biol Chem*. 2004;279:26509–26517.

59. Anantharamaiah GM, Mishra VK, Garber DW, et al. Structural requirements for antioxidative and anti-inflammatory properties of apolipoprotein A-I mimetic peptides. *J Lipid Res*. 2007;48:1915–1923.

60. Van Lenten BJ, Wagner AC, Jung CL, et al. Anti-inflammatory apoA-I-mimetic peptides bind oxidized lipids with much higher affinity than human apoA-I. *J Lipid Res* 2008;49:2302–2311.

61. Imaizumi S, Grijalva V, Navab M, et al. L-4F differentially alters plasma levels of oxidized fatty acids resulting in more anti-inflammatory HDL in mice. *Drug Metab Lett*. 2010;4:139–148.

62. Navab M, Anantharamaiah GM, Reddy ST, et al. Apolipoprotein A-I mimetic peptides. *Arterioscler Thromb Vasc Biol*. 2005;25:1325–1331.

63. Navab M, Anantharamaih GM, Reddy ST, et al. Oral D-4F causes formation of pre-β high-density lipoprotein and improves high-density lipoprotein-mediated cholesterol efflux and reverse cholesterol transport from macrophages and in apolipoprotein E-null mice. *Circulation*. 2004;109:3215–3220.

64. Ou J, Ou Z, Jones DW, et al. L-4F, an apolipoprotein A-1 mimetic, dramatically improves vasodilation in hypercholesterolemia and sickle cell disease. *Circulation*. 2003;107:2337–2341.

65. Ou J, Wang J, Xu H, et al. Effects of D-4F on vasodilation and vessel wall thickness in hypercholesterolemic LDL receptor-null and LDL receptor/apolipoprotein A-I double-knockout mice on Western diet. *Circ Res*. 2005;97:1190–1197.

66. Wool GD, Cabana VG, Lukens J, et al. 4F Peptide reduces nascent atherosclerosis and induces natural antibody production in apolipoprotein E-null mice. *FASEB J*. 2011;25:290–300.

67. Caligiuri G, Khallou-Laschet J, Vandaele M, et al. Phosphorylcholine-targeting immunization reduces atherosclerosis. *J Am Coll Cardiol*. 2007;50:540–546.

68. Binder CJ, Hörkkö S, Dewan A, et al. Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between *Streptococcus pneumoniae* and oxidized LDL. *Nat Med*. 2003;9: 736–743.

69. Tabet F, Remaley AT, Segaliny AI, et al. The 5A apolipoprotein A-I mimetic peptides displays antiinflammatory and antioxidant properties in vivo and in vitro. *Arterioscler Thromb Vasc Biol*. 2010;30:246–252.

70. Getz GS, Wool GD, Reardon CA. HDL apolipoprotein-related peptides in the treatment of atherosclerosis and other inflammatory disorders. *Curr Pharm Des*. 2010;16:3173–3184.

71. Navab M, Anantharamaiah GM, Hama S, et al. Oral administration of an apoA-I mimetic peptide synthesized from D-amino acids dramatically reduces atherosclerosis in mice independent of plasma cholesterol. *Circulation*. 2002;105:290–292.

72. Navab M, Anantharamaiah GM, Hama S, et al. D-4F and statins synergize to render HDL antiinflammatory in mice and monkeys and cause lesion regression in old apolipoprotein E-null mice. *Arterioscler Thromb Vasc Biol*. 2005;25:1426–1432.

73. Navab M, Reddy ST, Anantharamaiah GM, et al. The intestine maybe a major site of action for the ApoA-I mimetic peptide 4F whether the peptide is administered subcutaneously or orally. *J Lipid Res*. 2011; 52:1200–1210.

74. Van Lenten BJ, Navab M, Anantharamaiah GM, Buga GM, Reddy ST, Fogelman AM. Multiple indications for anti-inflammatory apolipoprotein mimetic peptides. *Curr Opin Investig Drugs*. 2008;9: 1157–1162.

75. Sherman CB, Peterson SJ, Frishman WH. Apolipoprotein A-I mimetic peptides. A potential new therapy for the prevention of atherosclerosis. *Cardiol Rev*. 2010;18:141–147.

76. Zhang Z, Datta G, Zhang Y, et al. Apolipoprotein A-I mimetic peptide treatment inhibits inflammatory responses and improves survival in septic rats. *Am J Physiol Heart Circ Physiol*. 2009;297: H866–H873.

77. Charles-Schoeman C, Banquerigo ML, Hama S, et al. Treatment with an apolipoprotein A-1 mimetic peptide in combination with pravastatin inhibits collagen-induced arthritis. *Clin Immunol*. 2008;127: 234–244.

78. Woo JM, Lin Z, Navab M, et al. Treatment with apolipoprotein A-1 mimetic peptide reduces lupus-like manifestations in a murine lupus model of accelerated atherosclerosis. *Arthritis Res Ther*. 2010;12:R93.

79. Bloedon LT, Dunbar R, Duffy D, et al. Safety, pharmacokinetics, and pharmacodynamics of oral apoA-I mimetic peptide D-4F in high-risk cardiovascular patients. *J Lipid Res*. 2008;49:1344–1352.

80. Watson CE, Weissbach N, Kjems L, et al. Treatment of patients with cardiovascular disease with L-4F, an apo-A1 mimetic, did not improve select biomarkers of HDL function. *J Lipid Res*. 2011;52:361–373.