The self-association of proteins to form amyloid fibrils has been implicated in the pathogenesis of a number of diseases including Alzheimer’s, Parkinson’s, and Creutzfeldt-Jakob diseases. We recently reported that the myeloid scavenger receptor CD36 initiates a signaling cascade upon binding to fibrillar β-amyloid that stimulates recruitment of microglia in the brain and production of inflammatory mediators. This receptor plays a key role in the pathogenesis of atherosclerosis, prompting us to evaluate whether fibrillar proteins were present in atherosclerotic lesions that could initiate signaling via CD36. We show that apolipoprotein C-II, a component of very low and high density lipoproteins, readily forms amyloid fibrils that initiate macrophage inflammatory responses including reactive oxygen production and tumor necrosis factor α expression. Using macrophages derived from wild type and Cd36−/− mice to distinguish Cd36-specific events, we show that fibrillar apolipoprotein C-II activates a signaling cascade downstream of this receptor that includes Lyn and p44/42 MAPKs. Interruption of this signaling pathway through targeted deletion of Cd36 or blocking of p44/42 MAPK activation inhibits macrophage tumor necrosis factor α expression. Finally, we demonstrate that apolipoprotein C-II in human atheroma co-localizes to regions positive for markers of amyloid and macrophage accumulation. Together, these data characterize a CD36-dependent signaling cascade initiated by fibrillar amyloid species that may promote atherogenesis.

CD36 is a multifunctional cell surface receptor expressed on macrophages, platelets, skeletal muscle, adipocytes, and microvascular endothelium (1). In myeloid cells, CD36 is believed to participate in the innate immune response through pattern recognition of foreign or modified-host ligands. As a result, this receptor has been reported to bind a diverse set of ligands including oxidized low density lipoprotein, thrombospandin-1, fatty acids, Plasmodium falciparum peptides, apoptotic cells, and most recently, β-amyloid (1). Although the physiological role of CD36 remains unclear, it has convincingly been shown to play a role in the genesis of atherosclerosis. In hyperlipidemic Apo-1−/− mice, targeted deletion of Cd36 resulted in a 75% reduction in atherosclerotic lesions in the aortic tree and a 45% reduction in lesion size in the aortic arch (2). The current hypotheses suggest that lipoproteins oxidized in the artery wall stimulate an influx of monocytes, which clear these toxic lipids via scavenger receptors, including CD36. These events are believed to precipitate the accumulation of lipid-engorged macrophage foam cells, the earliest histologic evidence of atherosclerosis. Studies using low density lipoprotein oxidized by several in vitro methods have shown that uptake of these ligands is severely impaired in human and mouse macrophages deficient in CD36 (3–6). However, despite intensive investigation, the lipids or proteins that trigger the influx of inflammatory cells into the artery wall remain poorly characterized and trials of antioxidant therapies have failed to show a positive impact on atherosclerosis (7, 8).

There is growing evidence that CD36 is linked to a signal transduction pathway that can initiate myeloid inflammatory responses that would be predicted to exacerbate atherosclerosis. We have shown that through binding of the fibrillar amyloid peptide, β-amyloid, CD36 initiates a pro-inflammatory signaling cascade that regulates microglial recruitment, activation, and secretion of inflammatory mediators (9, 10). In Alzheimer’s disease, the abnormal deposition of β-amyloid in the brain and vasculature incites chronic inflammation that contributes to neurotoxicity and cell death. The engagement of β-amyloid by CD36 results in the production of cytokines and chemokines implicated in this response including interleukin-1β, tumor necrosis factor α (TNFα), monocyte chemoattractant protein-1, and macrophage inflammatory protein-1α and β and macrophage inflammatory protein-2 (9–11). Interestingly, these inflammatory mediators have also been identified in human and mouse atherosclerotic lesions (12).

Despite extensive efforts, we have been unable to demonstrate macrophage CD36-specific signaling in response to in vitro oxidized low density lipoprotein. Thus, we hypothesized that atheroma may contain fibrillar amyloid proteins that

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1 The abbreviations used are: TNF, tumor necrosis factor; MAPK, mitogen-activated protein; PBS, phosphate-buffered saline; DMEM, Dulbecco’s modified Eagle’s medium; ROS, reactive oxygen species; SAP, serum amyloid P; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; LXR, liver X receptor.

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could initiate pro-inflammatory CD36 signaling in macrophages accumulating in the artery wall. One such candidate protein is apolipoprotein C-II (apoC-II), a component of very low and high density lipoproteins and a cofactor for lipoprotein lipase (13, 14). Apolipoprotein C-II is a 79 amino acid protein that readily forms amyloid fibrils under lipid-free conditions, adopting a cross-β-sheet structure that reacts with the amyloid stains thioflavin T and Congo Red (15). The ability of apolipoproteins to adopt this conformation is not limited to apoC-II but has also been reported for various full-length and mutant prototins to adopt this conformation is not limited to apoC-II stains thioflavin T and Congo Red (15). The ability of apolipoproteins, such as apoC-II, apoC-IV, and apoC-II-IV, to form amyloid fibrils in vitro has been demonstrated (16–22). Recently, the nuclear receptors LXRα and LXRβ, which are activated by oxysterols in cholesterol-loaded macrophage foam cells, were shown to induce expression of the apoC family, apoC-I, apoC-IV, and apoC-II-IV gene cluster. Up-regulated expression of this gene cluster in macrophage foam cells would be expected to result in the secretion of these apolipoproteins in the artery wall in a lipid-poor state amenable to amyloid fibril formation (23, 24).

We demonstrate that apoC-II amyloid fibrils, but not freshly prepared monomeric apoC-II, binds to CD36 and initiates a signaling cascade similar to that induced by β-amyloid. This CD36-specific signaling pathway involves the sequential activation of the Src kinase Lyn and the p44/42 MAPK. Wild type macrophages exposed to fibrillar apoC-II exhibit markers of macrophage activation including the production of reactive oxygen and TNFα gene expression, whereas macrophages lacking CD36 failed to respond. In human coronary arteries, apoC-II was detected in regions that stained positive for amyloid and macrophage markers. These results provide insight into the potential mechanism by which CD36 interaction with fibrillar amyloid proteins may promote inflammation in atherosclerosis.

MATERIALS AND METHODS

ApoC-II Preparation—ApoC-II was expressed and purified as described previously and stored as a stock solution at 30 °C in 5 mM GuHCl (15, 25). The protein was refolded by rapid dilution (1:100) from the stock solution into PBS, pH 7.4. For fibril formation, 0.3 mg/ml apoC-II was incubated at 20 °C for 72 h prior to use as described previously. Fibril formation was confirmed by thioflavin S (Sigma) fluorescent staining as described previously (9, 15). Fluorescein-labeled apoC-II was generated by incubating fluorescein-5-maleimide (Molecular Probes) with a previously characterized derivative of apoC-II tagged with a C-terminal cysteine residue (4 °C overnight) (26). The labeled samples were purified on a Sephadex G-25 column equilibrated with 5 mM GuHCl, and 20 mM Tris-HCl, pH 7.0, and fractions containing labeled protein were pooled and concentrated using Centricon YM-3 centrifugal filters (Amicon, Billerica, MA). The degree of labeling of apoC-II was determined to be 19% by measuring the absorbance at 492 nm and using an extinction coefficient of 83,000 M⁻¹ cm⁻¹. Fibril formation of fluorescein-labeled apoC-II was induced as described above.

Mice—The C57BL/6 (CD36-/-, CD36+/-M1) mice were generated in our laboratory as described previously and were backcrossed to C57BL/6J mice for 7 generations (9). Age-matched CD36-/- mice generated from crosses of CD36-/- mice were used as controls. Lyn-/- (Lyn<sup>−/−</sup>) and wild type littermate control mice were generated from intercrosses of Lyn<sup>−/−</sup> mice (The Jackson Laboratory). All of the mice were maintained in a pathogen-free facility with free access to rodent chow and water.

Cell Culture—Elicited peritoneal macrophages were collected by peritoneal lavage 4 days after intraperitoneal injection of 6–8-week-old mice with 3% thioglycollate as we described previously (9). Cells were washed in PBS, cultured for 2 h in DMEM containing 5% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml), and washed again to remove non-adherent cells. Adherent cells prepared in this manner were routinely >95% CD11b<sup>+</sup> and F4/80<sup>−</sup> as determined by flow-cytometric analysis. For signaling experiments, cells were incubated in DMEM with reduced fetal bovine serum (1%) overnight prior to use.

The Bowes human melanoma cell line was obtained from American Type Culture Collection (Manassas, VA) and was maintained in DMEM containing 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). A stable Bowes cell line expressing human CD36 was generated by co-transfecting the mammalian expression vector for human CD36, CDMS-CD8, and pcDNA-neomycin using calcium phosphate and selecting G418 sulfate-resistant cells as we described previously (27). A mock-transfected Bowes cell line was generated by co-transfection of the empty CDMS vector and pcDNA-neomycin. Expression of CD36 on transfected cells was detected by flow-cytometric analysis. Cells were incubated with an anti-human CD36 antibody (P4A9, 10 μg/ml) for 30 min, washed three times in PBS containing 1% bovine serum albumin, and incubated with fluorescein isothiocyanate-labeled rabbit anti-mouse IgG.

Western Analysis—Cells were washed in ice-cold PBS and lysed in radiodimmune precipitation buffer containing protease and phosphatase inhibitors, and 40–80 μg of protein was run on 10% denaturing SDS-polyacrylamide gels. Proteins were transferred to nitrocellulose membrane, blocked in 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20, and incubated overnight at 4 °C with primary antibody as we described previously (28). Primary antibodies included mouse anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology Inc., Lake Placid, NY), rabbit anti-phospho-p44/42, and rabbit anti-p44/42 antibody (Cell Signaling Technology, Beverly, MA). Blots were washed three times in Tris-buffered saline containing 0.1% Tween 20 and incubated with species appropriate horseshadish peroxidase-conjugated secondary antibody (1:1,000 dilution) for 1 h. Blots were washed three times in Tris-buffered saline containing 0.1% Tween 20 and exposed to ECL reagent (Amersham Biosciences), and signal was recorded using Kodak BioMax MR film or the Alpha Innotech Fluorchem 8800 image analysis software.

Measurement of Reactive Oxygen Species (ROS) Production—Reactive oxygen production was measured by nitro blue tetrazolium reduction assay as we described previously (9, 10). Cells (10<sup>5</sup>) were incubated on 6-mm<sup>2</sup> multispot slides in DMEM containing 1% fetal bovine serum for 1 h and stimulated with 10 μM fibrillar apoC-II or 40 μM fibrillar β-amyloid for 10 min at 37 °C. 50 μl of 1 μg/ml nitro blue tetrazolium was added, and cells were incubated for an additional hour at 37 °C. ROS production correlates with the formation of formazan, a dark blue-colored insoluble precipitate, the intensity of which was quantified by microscope video capture (5 measurements/sample) and ImageQuant analysis software.

Real-time Quantitative Reverse Transcriptase-PCR Analysis—Total RNA was extracted using TRIzol B reagent, and real-time quantitative reverse transcriptase-PCR was performed using the QuantiTect SYBR Green PCR kit (Qiagen, Valencia, CA) as we described previously (28). Each reaction contained 0.3 μM TAFs and glyceraldehyde-3-phosphate dehydrogenase primers and 5 μl of cDNA, SYBR Green, and HotStarTag polymerase. PCR was performed using a Bio-Rad iCycler under the following conditions: 15 min at 95 °C followed by 30 cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C. Each sample was analyzed in triplicate, and the amount of TAFs and glyceraldehyde-3-phosphate dehydrogenase mRNA in each sample was calculated from a standard curve of a known template.

Immunohistochemical Staining—Human left anterior descending coronary artery segments were obtained from hearts excised at the time of cardiac transplantation and then fixed in neutral buffered formalin and embedded in paraffin as described previously (29). Atherosclerotic plaques were identified by morphological criteria (30). Morphology was determined from 6-μm sections stained with Movat’s pentachrome stain. Macrophages were identified using monoclonal antibody HAM-56 (titer: 1:10, DakoCytomation, Carpinteria, CA) (31). Rabbit polyclonal antisera were used to identify apoC-II (titer: 1:500, Calbiochem), serum amyloid P (SAP) component (titer: 1:100, Calbiochem), or CDS6 (titer: 1:100, Calbiochem). Single label immunohistochemistry was performed using techniques described previously (29) with the exception that, following paraffin removal and rehydration through graded alcohols, sections intended for SAP immunostaining were pre-treated with Nova Red (DakoCytomation) according to the manufacturer’s protocol. Nova Red (Vector Laboratories, Burlingame, CA), which yields a red reaction product, was used as the peroxidase substrate, and cell nuclei were counterstained with hematoxylin.

RESULTS

The fibrillar conformation of apoC-II incubated under lipid-free conditions at physiological pH and salt concentrations was confirmed by visualization of fluorescence with the amyloid diagnostic dye thioflavin S as we described previously (Fig. 1) (9, 32). Purified apoC-II diluted in PBS, pH 7.4, and incubated for 72 h demonstrates thioflavin S fluorescence (Fig. 1a) that is not detected in freshly diluted samples (native apoC-II, Fig. 1b).
Amyloid Apolipoprotein C-II Promotes CD36 Signaling

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FIG. 1. ApoC-II amyloid fibrils activate p44/42 MAPK signaling. ApoC-II incubated at 20 °C to promote fibril formation (a) but not freshly prepared monomeric apoC-II (b) demonstrates thioflavin S fluorescence staining, confirming the presence of amyloid. c, Western blot demonstrating that fibrillar but not freshly prepared monomeric apoC-II induces phosphorylation of the p44/42 MAPK. Protein lysates from peritoneal macrophages stimulated with 10 μM fibrillar apoC-II or freshly prepared monomeric apoC-II were run on SDS-PAGE gels and immunoblotted using a phospho-specific p44/42 antibody (upper panel). The Western blot was stripped and re-probed with an antibody that detects total p44/42 protein (lower panel).

In this fibrillar conformation, apoC-II initiates p44/42 MAPK signaling in macrophages (Fig. 1c). Peritoneal macrophages treated with 10 μM fibrillar apoC-II demonstrate a rapid phosphorylation of p44/42 that peaked at 5–10 min post-stimulation. In contrast, no phospho-p44/42 protein was detectable in macrophages treated with freshly prepared monomeric apoC-II despite the presence of equivalent amounts of total cellular p44/42 in all cells (lower panel). These data indicate that apoC-II that has adopted a fibrillar amyloid conformation induces inflammatory signaling in macrophages.

Fibrillar ApoC-II Binds to CD36-expressing Bowes Melanoma Cells—We have recently shown that the macrophage scavenger receptor CD36 recognizes another amyloid protein, β-amylod, and initiates a cellular signaling cascade involving p44/42 MAPK. To test whether fibrillar apoC-II is a ligand for CD36, we assessed binding to human Bowes melanoma cells stably expressing CD36 (CD36-Bowes) using fluorescein-labeled apoC-II. Freshly prepared monomeric apoC-II fluorescein does not bind to CD36-Bowes cells as evidenced by the lack of cell surface fluorescence (Fig. 2a, right panel). By contrast, apoC-II-fluorescein allowed to form amyloid fibrils readily bound to the surface of CD36-expressing Bowes cells (Fig. 2, a, middle panel, and b, CD36 expression). In the absence of CD36, mock-transfected Bowes cells were unable to bind fibrillar apoC-II-fluorescein, indicating that binding is specifically mediated by this receptor (Fig. 2a, left panel).

CD36 Mediates Macrophage Inflammatory Responses to Fibrillar ApoC-II—Macrophage production of reactive oxygen species in response to inflammatory stimuli, including amyloid peptides, is a well-characterized marker of macrophage activation. We tested whether fibrillar apoC-II stimulated ROS production in peritoneal macrophages derived from wild type and Cd36−/− mice. In wild type macrophages, fibrillar apoC-II induced the generation of ROS (Fig. 3a). This was reduced by 75% in similarly treated Cd36−/− macrophages, indicating that the macrophage oxidative burst induced by fibrillar apoC-II requires CD36. Treatment with monomeric apoC-II did not stimulate ROS production in either macrophage population (data not shown). As we reported previously, fibrillar β-amylod also induced a CD36-dependent increase in cellular ROS (Fig. 3a). No difference was detected in the ROS response of wild type and Cd36−/− macrophages to another inflammatory stimulus, zymosan (data not shown). These data indicate that the binding of CD36 by unrelated amyloid proteins activates the macrophage respiratory burst.

To test whether fibrillar apoC-II induces expression of pro-inflammatory cytokines, we evaluated TNFα gene expression in wild type and Cd36−/− macrophages. Stimulation of wild type macrophages with 10 μM fibrillar apoC-II induced a 6-fold increase in TNFα mRNA as measured by quantitative real-time PCR (Fig. 3b). Similar treatment of macrophages lacking CD36 resulted in a small decrease in TNFα mRNA as compared with unstimulated macrophages. Freshly prepared monomeric apoC-II did not alter expression of TNFα in either macrophage population (data not shown). These data identify the production of TNFα as a macrophage response to fibrillar apoC-II and demonstrate that this is mediated by CD36.

Fibrillar ApoC-II Activates a CD36 Signaling Cascade Involving Phosphotyrosine, Lyn, and p44/42 MAPKs—Tyrosine kinase signaling is rapidly activated in macrophages by inflammatory stimuli, and thus, tyrosine phosphorylation has been used as a marker of signal transduction. Using macrophages derived from wild type and Cd36−/− mice, we investigated whether fibrillar apoC-II engagement of CD36 initiates phosphorylation of protein tyrosine residues. Fibrillar apoC-II induced the accumulation of five tyrosine-phosphorylated proteins in wild type macrophages that were not present or were greatly reduced in similarly treated Cd36−/− macrophages. Western blotting with an antibody directed against phosphotyrosine demonstrates the differential phosphorylation of these five proteins (approximate sizes: 42, 57, 75, 90, and 120 kDa) within 5–10 min post-stimulation (Fig. 4a). The smallest of these proteins is concordant in size with the p44/42 MAPK, which we found was activated by fibrillar but not monomeric apoC-II. Western blotting with an antibody to phospho-p44/42 revealed that activation of p44/42 MAPK by fibrillar apoC-II required the expression of CD36 (Fig. 4b). In Cd36−/− macrophages, the accumulation of phospho-p44/42 was no longer detectable 5 min post-stimulation and was greatly reduced at 10 min as compared with wild type macrophages. To eliminate the possibility that the signaling events observed could be the result of contamination of reagents with lipopolysaccharide, fibrillar apoC-II signaling was also assessed in macrophages lacking the lipopolysaccharide receptor, CD14, or lacking both CD14 and CD36. Phosphorylation of p44/42 in response to fibrillar apoC-II continued to be detected in Cd14−/− macrophages but was lost in Cd14−/−/Cd36−/− macrophages, confirming that CD36 is required for the initiation of fibrillar apoC-II signaling (Fig. 4b).

The Src family of tyrosine kinases can associate with CD36 and has been linked to the activation of MAPK. Pretreatment of macrophages with a broad inhibitor of Src kinases, PP1, abrogated p44/42 activation by fibrillar apoC-II. In the presence of this inhibitor, the accumulation of phospho-p44/42 was no longer detectable at 5 and 10 min post-stimulation and was greatly reduced at 20 min (Fig. 4c). Targeted deletion of the most abundantly expressed Src kinase family gene in macrophages, Lyn, blocked p44/42 phosphorylation in response to fibrillar apoC-II (Fig. 4d). This indicates that Lyn kinase plays an essential role in the CD36-mediated activation of p44/42 MAPK. Together, these data outline a signaling cascade initiated by fibrillar apoC-II in macrophages that involves the sequential activation of CD36, Lyn kinase, and p44/42 MAPK.

To determine whether the described signal transduction pathway regulates fibrillar apoC-II-induced TNFα expression in macrophages, we treated cells with the MEK inhibitor PD98059, which blocks activation of p44/42 MAPK (Fig. 5). As
expected, untreated macrophages or macrophages stimulated with monomeric apoC-II expressed little TNFα mRNA. Treatment of macrophages with fibrillar apoC-II for 12 h induced the expression of ~130 pg of TNFα mRNA. Pretreatment of macrophages with PD98059 reduced this increase in TNFα mRNA by ~60%. These data indicate that p44/42 MAPK signaling is responsible in part for fibrillar apoC-II activation of TNFα gene expression.

Co-localization of ApoC-II and Macrophages in Human Atherosclerotic Lesions—To investigate the physiological relevance of fibrillar apoC-II/CD36 signaling, human coronary atherosclerotic lesions were stained using antibodies specific for apoC-II, macrophages, and SAP, a universal constituent of amyloid deposits. As reported previously in murine aortic lesions, apoC-II was detected on a subset of intimal macrophages (33). All of the macrophages in this region expressed CD36 as detected by immunohistochemistry (data not shown). In addition, in the region adjacent to this large collection of macrophages, more widespread extracellular apoC-II staining was detected. Furthermore, this extracellular apoC-II co-localized with SAP, a marker of amyloid fibrils (Fig. 6, arrows). Together, these data are consistent with the hypotheses that apoC-II not only is localized in atherosclerotic lesions in regions of macrophage accumulation but also that apoC-II may form amyloid fibrils.

**DISCUSSION**

Monocytes and their macrophage counterparts play a critical role in the innate immune response through recognition of foreign or modified host proteins/lipids. This surveillance function is mediated in part by scavenger receptors, a family of pattern recognition receptors known to bind a diverse set of ligands including modified lipoproteins, proteins modified by advanced glycation endproducts, Gram-negative bacteria, apoptotic cells, and Plasmodium parasitized erythrocytes (34). Recently, we demonstrated that the class B scavenger receptor, CD36, binds β-amyloid and engages a signaling cascade in peripheral and brain macrophages that initiates inflammatory responses characteristic of those found in Alzheimer’s disease (9, 10). Amyloid fibrils of widely varying primary sequence can be recognized by a common antibody, suggesting a three-dimensional structure that exposes a shared epitope (35). In this report, we show that apoC-II, an unrelated plasma protein, readily forms amyloid fibrils that engage a similar CD36 signaling cascade involving Lyn and p44/42 MAPKs. Activation of this CD36 signaling pathway was shown to induce macrophage inflammatory responses including reactive oxygen production and TNFα. These data suggest that CD36 recognizes unrelated fibrillar amyloid proteins and engages a common signaling cascade that triggers the macrophage response to these toxic molecules.

CD36 has an established role in atherosclerosis (1). In the absence of this receptor, atherosclerotic lesion size in the Apoe−/− hyperlipidemic mouse is greatly reduced (2); however, whether this is attributable to its cholesterol uptake function or its ability to induce inflammatory signaling is not known. The current paradigm suggests a role for CD36 in binding oxidized lipoproteins in the artery wall. Despite a substantial...
literature suggesting that oxidation products of these endogenous lipids activate the chronic inflammatory state in atherosclerosis, trials of antioxidant therapies in humans have failed to show a protective effect for cardiovascular disease. Our recent finding that \( H9252 \)-amyloid activates a robust signaling cascade via CD36 led us to investigate whether proteins present in the artery wall that could form amyloid would engage this CD36 pathway. Apolipoproteins, normally associated with lipoprotein particles, can form amyloid fibrils that have been detected \textit{in vivo}. We identified a CD36 signaling cascade activated by amyloid fibrils of apoC-II, an apolipoprotein whose expression is regulated by oxysterols in macrophage foam cells. Immunohistochemical staining of human atheroma showed accumulation of apoC-II in regions positive for macrophage and amyloid markers. These findings raise the intriguing possibility that non-lipid ligands may initiate complicity of CD36 in...
atherogenesis by activating inflammatory signal transduction and suggest that the protein component of lipoproteins may contribute to atherosclerosis in a way that has been overlooked previously.

We employed human apoC-II in our studies because of the extensive characterization of its ability to form amyloid fibrils in vitro. In the absence of lipid, apoC-II forms twisted ribbon-like fibrils with all of the hallmarks of amyloid including binding to Congo Red and thioflavin T, increased β-sheet structure as measured by circular dichroism, and β-sheet formation as seen by x-ray diffraction (15, 32, 36). However, the ability of apoC-II to adopt an amyloid structure is not unique to this apolipoprotein and has also been reported for derivatives of apoA-I, apoA-II, apoA-IV, and apoE (16–21). Amyloid fibrils of apoA-I have been detected in plaques of both Alzheimer’s and atherosclerosis, and amyloid fibrils of a C-terminal apoE fragment is postulated to play a role in β-amyloid deposition and senile plaque formation (17–20). The formation of amyloid fibrils by apolipoproteins may be promoted by macromolecular crowding (30), dissociation of exchangeable apolipoproteins from lipoproteins (37), or displacement of apolipoproteins from the surface of lipoproteins by acute phase proteins such as serum amyloid A (32, 38). Furthermore, hypercholesterolemia has been suggested to upregulate macrophage expression of apolipoproteins in the artery wall through LXR-induced transcription of the apoE/C-I/IC-II gene cluster (24). Thus, activation of LXR by oxysterols in macrophage foam cells may result in increased levels of lipid-free apolipoproteins in the subendothelial space that would be amenable to amyloid conformation. Consistent with our finding in human atheroma, the authors of this study identified apoC-II in murine aortic lesions that co-localized with our finding in human atheroma, the authors of this study suggest that apoC-II may be induced in macrophage foam cells.

Studies evaluating the presence of amyloid in atherosclerotic plaque have reported the detection of amyloid fibrils in 16–59% of aortic lesions (17, 18). In addition to apolipoproteins, other proteins capable of forming amyloid have been detected in human atheroma that could engage CD36 signaling including β-amyloid and α1-antitrypsin (39–41). Immunohistochemical analysis of advanced human atherosclerotic plaques revealed the presence of β-amyloid peptide in activated perivascular macrophages expressing inducible nitric oxide synthase. In addition, a C-terminal cleavage product of the serum proteinase inhibitor α1-antitrypsin reported to form amyloid fibrils has been shown to induce monocyte inflammatory responses that can be blocked with CD36 antibodies in vitro (42). It seems likely that this α1-antitrypsin peptide engages the CD36 signaling cascade that we have described. Taken together, these data suggest that through recognition of amyloid proteins present in atherosclerotic lesions, CD36 may promote inflammation, re-enforcing the atherosclerotic effects of cholesterol.
